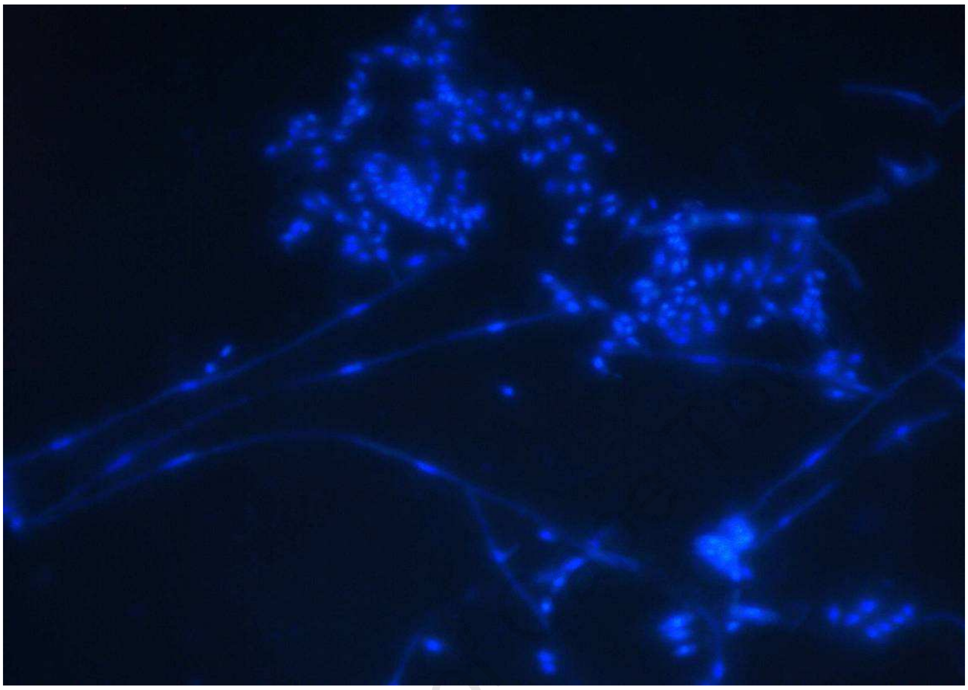
Microbial attachment to sulfide minerals in a bioleach environment



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Declaration

I know the meaning of plagiarism and declare that all the work in the document, save for that which is properly acknowledged, is my own.

4th November 2009

Cindy-Jade Africa

Dedication

A good friend once professed to me: "Art is a science; it is the science of freedom!"

This provoked a profound realization, that science is in fact an art!

More specifically (to me) it is the art of will, where will, in addition to one having strong resolve, encompasses the capability of conscious choice, decision and intention.

Art and science co-exist synergistically, creating a wealth and coalescence of freedom. Freedom of imagination, mental flexibility and fortitude!

Both (science and art) can be groomed,

Both can be beautiful,

And both can be painfully obscure!

I dedicate this work to all who are willing to take up the challenge and embrace the opportunity and their ability to be "free". I dedicate this to; those who taught me, those who instilled in me the importance of curiosity and a want for knowledge, those who nurtured and encouraged me and my enthusiasm for science, those whose faith in me made me believe in myself and my capability, those who inspired me to be and do more. For your help, encouragement and inspiration I will be forever grateful and I thank you.

Synopsis

This research pertains to bioleaching of copper containing ores with particular reference to the copper sulfide mineral chalcopyrite ($CuFeS_2$). While it is focused on heap bioleaching, it has applications to stirred tank bioleaching operations.

Industrial heap bioleaching offers opportunities for processing of low grade ores but poses process operational challenges. These challenges include ineffective heap inoculation, a lag period before effective leaching commences and poor heap performance. These aspects are attributed to several contributing factors, such as heap construction, engineering and microbial activity. To date little attention has been paid to colonisation as a means of mitigating these challenges and effectively improving process operation.

Current literature regarding microbial attachment to sulfide minerals is limited to pure culture studies using iron oxidising mesophiles, and the use of sulfide mineral concentrates. In a heap environment, mineral dissolution is accelerated through the presence of a mixed consortium of microbial species; with the contribution of each not yet fully understood. In addition, gangue minerals comprise the bulk of the minerals present and thus cannot be neglected when attempting to better understand microbial attachment and the role of microorganisms in a heap environment. The predominant methodology employed to study microbial attachment in a bioleach context has used batch agitated systems (shake flasks). This may not adequately represent attachment under heap-like fluid dynamics.

The idea of this project stemmed from a requirement to contribute to the mitigation of challenges faced by industry through addressing the aforementioned gaps prevailing in literature and improving understanding of the role of microbial attachment and colonisation under conditions simulating a heap. The aim of this study was to investigate attachment of three bioleach microorganisms (*A. ferrooxidans, L. ferriphilum* and *S. metallicus)* to complex, sulfide-containing minerals ores in a bioleach environment using methodologies simulating heap-like conditions.

Three approaches were employed:

- Pure culture, agitated batch systems using shake flasks
- Pure culture, continuous fluid flow systems using packed column reactors
- Pure and mixed culture, continuous flow through systems using a biofilm reactor

In the first approach, an agitated batch assessment of the trends in microbial attachment to sulfide minerals was compared to the quartz control mineral. In batch agitated systems, *A. ferrooxidans* was used and experiments were conducted in Erlenmeyer shake flasks containing a 2 % wt vol^{-1} of the mineral of interest allowing a microbe-mineral contacting time ranging from 1 to 120 minutes. High levels of attachment were attained to sulfide minerals, with 92 % and 97 % attachment to pyrite and chalcopyrite respectively, and 52 % and 50 % attachment to low grade chalcopyrite containing mineral ore and quartz respectively. Attachment was thus observed to be selective to sulfide minerals over low grade chalcopyrite mineral containing ore and quartz. Hydrodynamic conditions experienced in batch agitated systems differed from those in a heap. The findings of the agitated batch attachment study, though indicative of selective attachment of *A. ferrooxidans* to sulfide minerals, do not represent attachment dynamics in a heap environment adequately. Hence more representative techniques of investigating attachment (relevant to heap bioleaching) quantitatively were developed.

The packed column approach simulates heap-like fluid dynamics, an important factor overlooked in batch agitated systems to date, and has been demonstrated to be an effective means of investigating attachment under heap-like conditions. Three identical glass packed column reactors, each loaded with 300 mineral coated beads and operated as continuous flow systems, were used to investigate the effects of culture conditions on the degree of attachment of *A. ferrooxidans*, *L. ferriphilum* and *S. metallicus* to four different surfaces, namely: pyrite mineral concentrate, chalcopyrite mineral concentrate, low grade chalcopyrite containing mineral ore, and quartz. Equilibrium attachment was observed after two residence times (approximately 135 minutes).

The effect of growth history on cell surface charge and subsequent attachment ability was assessed using the packed column attachment approach. Cultures grown on solid sulfide mineral concentrate exhibited enhanced attachment to sulfide minerals over ferrous grown cells. Growth conditions affected cell surface charge. Pyrite mineral concentrate-adapted cultures exhibited a positive cell surface charge and ore free cultured microorganisms exhibited a negative cell surface charge. The growth conditioning of cultures using pyrite mineral adaptation and subsequent change in cell surface charge correlated to enhanced attachment efficiencies observed for all mineral adapted mesophilic cultures to sulfide mineral concentrates relative to ore free cultures.

Selective attachment was demonstrated using the packed column reactor system, with attachment observed to be preferential to sulfide mineral concentrates over the control quartz as lower attachment efficiencies were observed for the attachment of mesophilic microorganisms to low grade chalcopyrite mineral containing ore and the control mineral quartz, relative to the sulfide mineral concentrate systems. For mineral-adapted *A.*

ferrooxidans, attachment efficiencies of 74 ± 3.8 %, 63 ± 1.1 %, 25 ± 7.9 % and 23 ± 11.3 % were observed for the attachment to pyrite, chalcopyrite, gangue containing chalcopyrite mineral ore and quartz respectively; with 58 ± 2.6 %, 79 ± 2.6 %, 50 ± 4.1 % and 25 ± 7.2 % to each respective mineral system by mineral-adapted *L. ferriphilum*. For ore free cultured *A. ferrooxidans*, attachment efficiencies of 52 ± 4.3 %, 52 ± 1.5 %, 7 ± 4.7 % and 35 ± 0.8 % were observed to pyrite, chalcopyrite, gangue containing mineral ore and quartz respectively; with 55 ± 1.0 %, 27 ± 3.4 %, 26 ± 1.5 % and 51 ± 4.5 % observed to the respective minerals by ore free cultured *L. ferriphilum*. The microorganisms exhibited similar trends and affinities for attachment to the sulfide mineral concentrates.

Using the column approach, degrees of attachment was investigated to the four mineral systems using the thermophile *S. metallicus* at ambient conditions. Wash out was observed after 1.5 residence times (90 minutes). It was concluded that although data are relevant to the start up phase of a heap bioleach where temperatures are ambient, temperatures were sub-optimal for this microorganism and thus may underestimate the maximum levels of attachment attainable for *S. metallicus*. Selective attachment to sulfide mineral concentrates relative to the low grade chalcopyrite containing ore and the quartz control was observed. Attachment efficiencies observed for *S. metallicus* were lower than that observed for mesophiles.

The final approach successfully demonstrated the visualisation and investigation of attachment to pure and low grade chalcopyrite mineral containing ore thin sections, using pure and mixed cultures, under fluid velocities simulating that of a heap, *in situ*. A biofilm reactor, consisting of a circular vessel (10 cm outer diameter) to house a section of mineral ore was developed. Thinly sectioned ore mounted on glass slides, were mineralogically mapped using ore microscopy and photographic analysis. The use of low grade (gangue containing) chalcopyrite mineral ore was used to study attachment under conditions more representative of a heap. Once colonised, the thin sections were removed from the reactor and the attached cells visualised through the use of fluorescence and ore microscopy techniques. The novel technique allowed for the effective investigation of microbial ecology with special regard for microbe-mineral association, site and mineral specific attachment of microorganisms and spatial organisation of microbial communities. The density of attached microbial populations increased with an increase in contact time. Mono-layered biofilms were observed. Attachment of mixed microbial micro-colonies were observed in regions where surface defects were predominant and were also observed to be prominent in, but not limited to, regions containing sulfide minerals.

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ABBREVIATIONS

AFM	Atomic force microscopy
B	Bacteria
BS	Irreversible attachment
[BS]*	Reversible attachment and formation of the metastable complex
CSTR	Continuous stirred tank reactor
DAPI	4’, 6-diamidino-2-phenylindole
EPS	Extra-cellular polymeric substance
FISH	Fluorescent *in situ* hybridisation
PCR	Polymerase chain reaction
PPL	Plane polarised light
QEMSCAN	Quantitative evaluation of minerals by scanning electron microscopy
RL	Reflected light
rRNA	ribosomal ribonucleic acid
S	Surface sites involved in the initial adhesion mechanism
SEM	Scanning electron microscopy
TL	Transmitted light
XDLVO	Extended Derjaguin Landau Verwey Overbeek theory
XPL	cross polarised light
XRD	X-ray diffraction
XRF	X-ray fluorescence

NOMENCLATURE

C	Eluted cell number
C_0	Inoculum cell number
d	Depth of Thoma counting chamber (0.02 mm)
ΔG	Gibbs free energy
n	Number of observations
N	Number of squares enumerated using a Thoma counting chamber
t	Time (h)

Chapter 1: Introduction

Bioleaching is an application of biotechnology exploited by the mining industry for the recovery of metals from low-grade ores. Acidophillic, chemolithotrophic microorganisms, which may occur naturally in the soil environment, catalyse the dissolution of metals from minerals. The mineral dissolution is due to a chemical attack of ferric iron, or protons, or both on the mineral surface. This results in the formation of ferrous iron and various forms of sulphur. The microorganisms involved in the process oxidize ferrous iron and reduced sulphur compounds to gain energy, forming ferric iron and sulphuric acid. Thus, involved effectively regenerate the reactants required for mineral dissolution.

Bioleaching has been exploited successfully for the recovery of a variety of metals including gold, cobalt and copper (Rawlings 2004). This research will pertain to bioleaching of copper containing ores with particular reference to the copper sulfide mineral chalcopyrite ($CuFeS_2$) as primary[1] chalcopyrite comprises the bulk source of copper available for metal recovery (Peters *et al.* 1966). Industrial bioleaching operations may be carried out in stirred tank reactors or via the construction of heaps. This research is focussed on heap bioleaching, while having application to stirred tank bioleaching operations.

Bioleach heaps are constructed using agglomerated ore. The heaps are aerated from the bottom and an acidic medium is percolated through the heap from the top and collected in reservoirs at the base. The collected medium is known as the pregnant leach liquor is then sent for metal recovery (Rawlings 2004). Although the microorganisms involved may occur naturally on the ore, inoculation of the heap enhances initial dissolution rates (Rawlings 2004) and ensures that the microbial flora necessary for effective leaching at various conditions is present. Heap bioleaching is generally a more economical means of recovering copper from low-grade ores. This method of bioleaching is also beneficial for the recovery of metals from ores that, if treated using conventional mining methods, would be seriously detrimental to the environment (Rawlings *et al.* 2003).

Despite the success of heap bioleaching for the economical recovery of copper, the process has succumbed to numerous problems. The 'mineralogy of ores and concentrates impacts directly on the efficiency of leaching and bioleaching and on the nature of the reaction products' (Watling 2006), which in turn affects bioleaching. Leaching of chalcopyrite in particular is problematic in comparison to other sulfide minerals (Stott *et al.* 2003). It is proposed that the problems associated with chalcopyrite may be due to the '*passivation of chalcopyrite'*.

[1] Primary minerals refer to minerals which have not been chemically altered in any way since their time of crystallisation from molten larva and subsequent deposition

Other problems associated with copper heap bioleaching include start-up difficulties, such as ineffective inoculation (Gericke *et al.* 2005), unpredictable lag periods experienced before the onset of efficient bioleach rates, as well as poor heap performance due to incomplete leaching of heaps. Addressing the prevailing difficulties with current heap bioleach operations is a strong motivation for the necessity of this research. The issues associated with heap bioleaching of copper sulfide minerals underscore the need for of knowledge with respect to microbial attachment and colonisation within a heap environment. Effective inoculation and successful colonisation of required microbial consortia throughout the heap is essential for increased initial dissolution rates, and therefore, a decreased lag period before effective bioleaching occurs; as well as for overall effective bioleaching of copper sulfide minerals (Gericke *et al*. 2005). Knowledge on how the prevailing microbial consortia changes as the leach progresses, with respect to microbial succession is required. Understanding the dominant microbes under various conditions may assist the predictability of bioleach operations. This may provide a certain level of control over the process as heaps can be inoculated with particular microorganisms under certain conditions which may enhance the bioleach rate.

The adherence of bacteria to surfaces is important, with respect to the microbial ecology, for the creation of favourable microenvironments, effective microbial colonisation, and biofilm formation. With respect to bioleach environments, attachment is believed to play an important role in the dissolution process. This involvement may be explained by the 'indirect mechanism' (Crundwell 2003). The mechanism suggests that the excretion of an extra-polymeric substance or EPS, which mediates irreversible attachment and biofilm formation, provides the reaction space for mineral dissolution to occur via the chemical attack previously mentioned (Harneit *et al.* 2005, Kinzler *et al.* 2003, Crundwell 2003). The EPS brings the reactants and the microbial catalysts in close proximity to the mineral surface, which facilitates a more effective chemical attack (Rawlings 2004). However, the exact role played by the microorganisms, with respect to the involvement of attachment in the bioleaching process, is still under debate.

Knowledge on the interactions between microbial and mineral surfaces, subsequent attachment of microorganisms to mineral surfaces, and the factors influencing these interactions will provide valuable information on the mechanism and role of microbial attachment, microbial colonisation and to an extent microbial succession within bioleach environments. The purpose of the research is to investigate the attachment of microorganisms commonly isolated from bioleach environments to sulfide mineral surfaces taking into account mineralogy. Consequently, this project serves to address the need for a further understanding of microbial colonisation in heap leach environments as well as the understanding of the bioleach process and the role played by micro-organisms therein.

The objectives of the study are the following:

- To review the role of microorganisms in bioleaching and factors affecting the bioleach process, as well as the available literature regarding the attachment of microorganisms to mineral surfaces and the mechanism of attachment processes
- To investigate trends in microbial attachment of *A. ferrooxidans, L. ferriphilum* and *S. metallicus* to a pyrite mineral concentrate, chalcopyrite mineral concentrate, low grade chalcopyrite mineral containing ore, using quartz as an inert control mineral in batch agitated and particle packed column reactors and validate the approaches used and assess the applicability of these approaches in a heap environment
- To assess the effects of growth history on surface charge and subsequent trends of microbial attachment to sulfide minerals using the particle packed column reactor
- To demonstrate and validate the development of a novel integrated *in situ* technique for the study of microbial attachment using the biofilm reactor
- To integrate conclusions on trends in microbial attachment observed across various methodologies

The literature review, presented in Chapter 2, contains an overview of bioleaching and the factors affecting this process. A review of microbial attachment to mineral surfaces and the mechanisms involved in the processes, trends in microbial attachment to sulfide minerals, as well as the effect of growth history on microbial attachment are presented. The advantages of attachment and the phenomenon of detachment are also presented. Chapter 3 presents the description of, and rationale behind, the methodologies employed in this study. All analytical techniques and reactor configurations are presented. Chapter 4 presents the results and discussion of the batch agitated attachment study through the use of shake flasks. Attachment efficiencies observed using this method for the attachment of *A. ferrooxidans* to sulfide minerals with quartz used as a control are presented and contrasted with literature reports. Chapter 5 presents the results and discussion of the particle packed column reactor attachment study. Attachment trends and efficiencies observed for *A. ferrooxidans, L. ferriphilum* and *S. metallicus* cultured in the presence or absence of solid sulfide mineral ore, to sulfide mineral concentrates, or a low grade chalcopyrite containing mineral ore, with quartz used as a control is presented and contrasted with literature reports. Chapter 6 presents the results and discussion of the biofilm reactor attachment study. Trends in attachment and colonisation of pure and mixed cultures of *A. ferrooxidans* and *L. ferriphilum* to a predominantly chalcopyrite containing mineral ore thin section, and a low grade chalcopyrite containing mineral ore thin section is presented. Chapter 7 presents a summary of conclusions drawn and recommendations made from this study.

Chapter 2: Literature review

2.1 AN OVERVIEW OF BIOLEACHING

The world copper production has increased steadily from 9 Mt to 16 Mt per annum from 1984 to 2005 (Watling 2006). Due to this growing world demand for copper, there is a continuous need for the minerals industry to find innovative means of economically maximising the product extracted. Bioleaching is one such mechanism making use of microorganisms for the recovery of metals from low grade copper ores, overburden and waste from existing operations. In this section a brief overview of the abiotic leach chemistry of copper sulfide minerals is provided (Section 2.1.1). This is followed by the introduction of the concept of microbially assisted 'bioleaching', in Section 2.1.2. Finally, the role of microbes in bioleaching and the mechanisms currently proposed are discussed in Section 2.1.3.

2.1.1 Abiotic leach chemistry for the dissolution of copper sulfide minerals

Leaching of sulfide minerals occurs in an acidic medium, containing ferric iron (Watling 2006). The process is oxidative, with ferric ion acting as the oxidant. Ferric ions oxidise the sulfide component of the mineral to elemental sulfur and during this redox reaction, the ferric ions are reduced to ferrous ions. Equation 1 below shows the oxidation of chalcopyrite by ferric ion. In addition to the oxidative process described previously, a non-oxidative reaction, shown in Equation 2 below, occurs which contributes to the overall mineral dissolution (Watling 2006).

$$CuFeS_2 + 4Fe^{3+} \longrightarrow 5Fe^{2+} + Cu^{2+} + 2S^0 \qquad \text{Eqn (1)}$$

$$CuFeS_2 + 4H^{+} \longrightarrow Fe^{2+} + Cu^{2+} + 2H_2S \qquad \text{Eqn (2)}$$

2.1.2 Microbially assisted leaching

The chemistry of microbially assisted leaching is similar to that of the abiotic chemical leaching due to the oxidation of sulfide mineral by ferric ions in acidic media as seen in Equation (1). Bioleaching uses chemolithoautotrophic micro-organisms to catalyse the dissolution of metals from minerals. This dissolution is due to a chemical attack of ferric iron or protons or both on the mineral surface. Microbes oxidize ferrous ion to ferric ion, and oxidize reduced sulfur compounds to sulphate, generating acid (Watling 2006). The microorganisms effectively catalyse the leaching process as they regenerate the reactants required for mineral dissolution to continue (see the indirect mechanism explaining microbial involvement in Section 2.1.3). The reactions can be seen below in Equation 3 and 4 respectively.

$$2Fe^{2+} + 2H^{+} + 0.5O_2 \longrightarrow 2Fe^{3+} + H_2O \qquad \text{Eqn (3)}$$

$$2S + 3O_2 + 2H_2O \longrightarrow 2H_2SO_4 \qquad \text{Eqn (4)}$$

2.1.3 Mechanisms to describe microbial involvement in bioleaching

There are currently three schools of thought explaining the involvement of microorganisms in bioleaching with arguments supporting all three. However, some of these debates lack validity and experimental evidence. The proposed mechanisms of microbial involvement have been summarised by Crundwell (2003), and termed: the **indirect mechanism**, **the direct contact mechanism** and the **indirect contact mechanism**.

In explaining the role of bioleach microorganisms via the **indirect mechanism**, it is hypothesised that the microorganisms regenerate the reactants required for the leaching process to continue through oxidation of iron sulfides to ferric iron and sulfuric acid. No contact is made by the microorganism with the mineral surface, and mineral dissolution occurs by chemical reaction in the presence of reactants (ferric ions and or protons) in solution.

According to Rawlings (2004), the **direct mechanism** of bioleaching involves microbes adhering to the mineral surface and some form of an enzymatic attack, with components of the membrane of the microbe interacting directly with the mineral surfaces. This was first proposed by Silverman and Ehrlich (1964), who hypothesised that an increased rate of pyrite dissolution by *Thiobacillus ferrooxidans* (currently classified as *Acidithiobacillus ferrooxidans*) over abiotic chemical dissolution was due to the involvement of some enzymatic oxidant (Crundwell 2003). Thus, in the case of the direct mechanism, attachment of microorganisms to the mineral surface is essential to play an active role in the leaching process, as mineral dissolution is a result of an enzymatic attack on the mineral surface. Crundwell (2003) notes that, to date, there is no evidence to support these claims, and many authors have made claims to contradict this behaviour of *Acidithiobacillus ferrooxidans* concerning pyrite dissolution. According to Crundwell (2003), the primary distinguishing feature between the indirect and direct mechanism is not the necessity for attachment but rather the requirement of ferric ions and protons for the dissolution of mineral via chemical attack in the indirect mechanism; and the involvement of an enzymatic attack in the direct mechanism for which, to date, experimental evidence in absent.

With respect to the **indirect contact mechanism**, the dissolution of the mineral is due to the chemical attack of ferric ions or protons or both on the mineral sulfide. This reaction gives rise to the formation of ferrous iron and various forms of sulfur (Rawlings 2004). The microbes involved obtain their energy by oxidation of ferrous, sulfur and reduced-sulfur compounds (Devasia *et al.* 1993). The role of microorganisms, via this mechanism, is to catalyse the oxidation of iron sulfides to ferric iron and sulfuric acid and thus regenerate the reactants required for the leaching process to continue. For this mechanism of bioleaching, microbes need not be in direct contact with the mineral surface in order for mineral dissolution to occur. Instead, it is believed that the extra-polymeric substance (EPS) produced by cells for attachment to surfaces and establishment of biofilms (discussed in Section 2.4), provides the reaction space for dissolution to occur (Gehrke *et al.* 1998, Harniet *et al.* 2005) in which reactants are in close proximity to the mineral surface facilitating a more effective chemical attack (Rawlings 2004). Therefore, with respect to the indirect contact mechanism, direct contact is not a requirement for bioleaching, however, attachment and biofilm formation plays an important role in bringing the microbial catalysts, the reactants and the mineral surface in close proximity, to facilitate a more effective dissolution process.

A schematic representation of the mechanisms used to explain the role of microorganisms in bioleaching is presented in Figure 2.1.

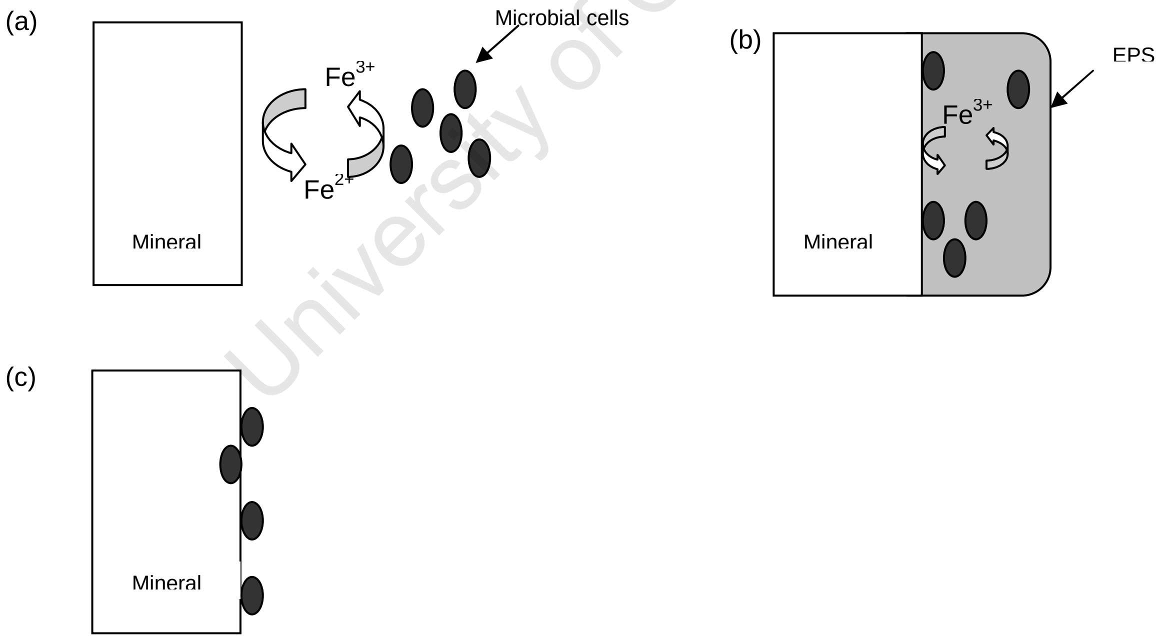

Figure 2.1: The role of microorganisms explained by the indirect mechanism **(a)**, mineral dissolution occurs due to the presence of ferric ions and or protons in solution. These reactants are regenerated by microbes via oxidation of ferrous iron and reduced sulfur compounds. In the indirect contact mechanism **(b)**, mineral dissolution is still due to the chemical attack of ferric ions and or protons in solution. The attachment and excretion of EPS by the microbes allows a more effective chemical attack by contacting of reactants, the mineral surface and the microorganisms. The direct contact mechanism **(c)** is depicted, with microbes interacting directly with the mineral surface. (Adapted from Crundwell 2003).

It is important to note that although attachment plays an essential role in both the direct and indirect mechanisms of bioleaching, the distinguishing difference lies in the mechanism mineral dissolution being a result of enzymatic attack (direct) or chemical attack by ferric ions and protons (indirect). While the activity of planktonic cells in the bioleach can not be ignored, microbial attachment is key in the leaching process and has been demonstrated to increase leach rates (Rodriguez *et al.* 2003 **a**), discussed in Section 2.5. In appreciation of this correlation, attachment is key in the development of a suitable hypothesis to describe microbial involvement in bioleaching. For the purpose of this research, the indirect contact mechanism will be used as a basis for understanding microbial involvement in bioleaching.

2.2 MICRO-ORGANISMS INVOLVED IN BIOLEACHING

Bioleach heaps present complex and varying mineralogical compositions, as well as environments, of extreme pH and temperature. Consequently, the microbial consortia isolated from these environments responsible for mineral dissolution, comprise an array of robust acidophilic (optimal pH for growth less than pH 3) bacteria and archaea. These microorganisms are iron and sulfur oxidizing chemolithotrophs. They obtain energy from the oxidation of ferrous iron to ferric iron, or the oxidation of reduced sulfur compounds with the formation of acid (Stott *et al.* 2003). The microorganisms isolated from heap leach environments may be mesophilic, moderately thermophilic and extreme thermophiles. Summarised in Table 2.1, are the bacteria and archaea commonly isolated from bioleach heap operations.

Only a few organisms have been isolated and to date not much is known with respect to how these consortia change as the leach progresses. Under mesophilic conditions *Leptospirillum ferriphilum* is proposed as the dominating microbial species. In continuous culture, at temperatures between 40 and 45℃ (moderately thermo philic conditions), *Acidithiobacillus caldus* is the dominant sulfur oxidiser in bioreactors treating arsenopyrite or copper concentrates (Rawlings *et al.* 1999, Okibe *et al.* 2003). At temperatures greater than 60℃, *Sulfolobus metallicus* (referred to as *S.metallicus* from now on) and *Metallosphaera spp.* are proposed as the dominating bioleaching strains (Hallberg and Johnson, 2001).

Table 2.1: List of microorganisms commonly isolated from bioleach environments (Taken from Watling 2006).

Organism	Reported growth substrates	Characteristics
Acidianus ambivalens	S oxidation and reduction	Hyperthermophiles
Acidianus brierleyi	Sulphides	pH opt 1.5–2.5
Acidianus infernus	Poor, if any, Fe oxidation	
"Acidianus tengchongensis "		
Acidimicrobium ferrooxidans	Mixotroph	Moderate thermophile pH opt 2
	Fe oxidation and reduction	
	Sulphides (poor)	
Acidiphilum spp	Obligate heterotrophs	Mesophiles
Acidiphilium SJH	S oxidation	pH opt ~ 2–3
	Fe(III) reduction	
Acidiphilium acidophilum	Facultative autotroph	Mesophile pH opt ~ 2–3
	S oxidation	
	Fe(III) reduction	
Acidithiobacillus albertensis	Autotrophs	Mesophiles
Acidithiobacillus ferrooxidans	S oxidation, sulphides	pH range 2–4
Acidithiobacillus thiooxidans	(*Af*, Fe(II) oxidation; Fe(III) reduction as a facultative anaerobe)	
Acidithiobacillus caldus	Mixotroph	Moderate thermophile
	3S oxidation, sulphides	pH opt 2–2.5
Acidolobus aceticus	Heterotroph	Hyperthermophile
	S reduction to H_2S	pH opt 3.8
Alicyclobacillus spp	S oxidation, sulphides	Mesophiles — moderate thermophiles
"Alicyclobacillus disulfidooxidans"	(*Ad*, facultative autotroph,;	pH 1.5–2.5
"Alicyclobacillus tolerans"	At, mixotroph, Fe(III) reduction)	
"Ferrimicrobium acidiphilium"	Heterotroph	Mesophile
	Fe(II) oxidation, sulphides	pH opt 1.7–1.8
	Fe(III) reduction	
Ferroglobus placidus	Fe oxidation	Thermophile
		pH neutral
"Ferroplasma acidarmanus"	Possibly autotroph	Moderate thermophiles
"Ferroplasma cyprexacervatum"	Iron oxidation	pH range < 1–2
Ferroplasma acidophilum	Pyrite oxidation poor	
Ferroplasma MT17		
Hydrogenobaculum acidophilus	S, H oxidation to produce sulphuric acid	Thermophile
		pH opt 3–4
Leptospirillum ferriphilum	Fe oxidation	Mesophiles, some thermo-tolerant strains
Leptospirilum thermoferrooxidans	Pyrite	pH range 1.6–1.9
Leptospirillum ferrooxidans	Fe oxidation, pyrite	Mesophile
		pH opt 1.5–1.7
Metallosphaera sedula	S oxidation	Thermophiles
Metallosphaera prunae	Sulphides	pH 1–4
"Metallosphaera hakonensis"		
Sulfobacillus acidophilus	Fe(II) oxidation; Fe(III) reduction, Sulphides	Moderate thermophiles
Sulfobacillus thermosulfidooxidans	S oxidation	pH 1–2.5
Sulfolobus metallicus	Strict chemolithoautotroph	Hyperthermophiles
"Sulfolobus rivotincti"	S oxidation, sulphides	Various pH in range 1–4.5
Sulfolobus shibatae		
"Sulfolobus tokodaii		
Sulfolobus yangmingensis		
"Sulfolobus" JP2 and JP3		
Sulfolobus acidocaldarius	Heterotrophs	Hyperthermophiles
Sulfolobus solfataricus	Not S oxidation	pH 2–4.5
Sulfurococcus yellowstonensis	S and Fe oxidation	Hyperthermophile
Thiobacillus prosperus	S and Fe oxidation	Mesophile, halophile
	sulphides	pH opt 2
Thiomonas cuprina	S oxidation, sulphides	Mesophile
		pH opt 3–4

2.3 MINERALOGY AND ITS EFFECTS ON HEAP BIOLEACHING

Mineralogical composition is complex, as the mineralogy changes with geological location and the constituent minerals react as the leach progresses forming various products which have been observed to affect bioleaching. Therefore, mineralogy of ores and concentrates has direct impact on the efficiency of leaching and bioleaching and on the nature of the reaction products (Watling 2006). However, to date, few quantitative studies of mineralogical effects with respect to leach chemistry and leach rates have been conducted (Watling 2006). The effects of ore mineralogy on bioleaching are presented in Section 2.3.1, with the techniques currently available for mineralogical analysis of ores and leach residues discussed in Section 2.3.2.

2.3.1 Effect of Mineralogy on bioleaching

Mineralogy is a key parameter in mining operations for the development of flow sheets, feasibility studies and predicting the efficiency of the operation (Watling 2006). It is important to note that mineralogical composition varies with the location of the mine, depending on how the copper ore body was formed and the extent of weathering experienced. Here the effects of the metal sulfide mineral content (Section 2.3.1.1) and the gangue mineralogy (Section 2.3.1.2) on bioleaching is discussed.

2.3.1.1 The effect of metal sulfides on heap bioleaching

Copper is usually deposited as copper sulfide minerals, with primary[1] chalcopyrite comprising the bulk source of copper available for metal recovery (Peters *et al.* 1966). Chalcopyrite leaching has proven to be challenging in comparison to other sulfide minerals (Stott *et al.* 2003). Chalcopyrite has also been shown to leach poorly at low temperatures (Rawlings *et al.* 2003). It is proposed that the problems associated with chalcopyrite may be due to the '*passivation of chalcopyrite'*, discussed later. Minerals commonly associated with chalcopyrite include: covellite, cubanite, enargite, galena, sphalerite, and pyrite. Secondary[2] bornite and chalcocite are also common forms of recoverable copper associated with chalcopyrite.

The biotic and abiotic leach chemistry for chalcopyrite has been discussed briefly in Section 2.1. However, the dissolution reaction was observed to be different for various metal sulfides, with acid soluble and acid insoluble metal sulfides undergoing oxidation via two distinct

[1] Primary minerals refer to minerals which have not been chemically altered in any way since their time of crystallisation from molten larva and subsequent deposition
[2] Secondary minerals are the result of the decomposition of a primary mineral and subsequent re-precipitation, creating a new, chemically distinct mineral

mechanisms and through dissimilar intermediates. Schippers and Sand (1999) proposed that acid insoluble metal sulfides, such as pyrite, are oxidized via the *thiosulphate mechanism* and acid soluble metal sulfides such as chalcopyrite, sphalerite and galena, undergo oxidation via the *polysulfide mechanism*. The *thiosulphate mechanism* involves oxidation by ferric attack on the mineral surface, with thiosulphate being the main intermediate and sulphate the main end product. In the *polysulfide mechanism* the dissolution is due to a combined oxidative attack of protons and ferric iron, with polysulfide as the major intermediate and elemental sulfur as the final product. The elemental sulfur can then be further oxidized to sulphate by microbial action.

The dissolution of mineral via the *polysulfide mechanism* is acid consuming, resulting in an increase in pH. Optimal microbial leaching occurs between pH 1.4 and pH 1.6 (Rawlings 2004). Over prolonged periods of bioleaching the increase in pH will hinder microbial leaching and also may lead to formation of precipitates. With respect to chalcopyrite dissolution in particular, the formation of basic sulphates such as jarosite on the mineral surface has been implicated in hindering complete chalcopyrite dissolution (Klauber 2003, in Watling 2006; Petersen *et al.* 2001).The phenomenon has been coined the '*passivation of chalcopyrite'* and may explain the difficulties associated with chalcopyrite leaching. High redox potential (0.65 - 0.70 SHE) due to the efficiency of ferrous to ferric oxidation by microorganisms was also found to be less favourable for complete chalcopyrite dissolution (Watling 2006). High redox potential also results in the precipitation of ferric ion to jarosite when in the vicinity of monovalent alkali cations and sulphate ions (Watling 2006). Formation of jarosite precipitates was proposed to be circumvented by operating leach conditions at pH 1 (Kinnunen *et al.* 2003). Fowler and Crundwell (1999) observed that oxidation of the sulfur product layer by *A. ferrooxidans* increases the rate of sphalerite dissolution, thus the formation of a sulfur layer on mineral surface does not limit the sphalerite dissolution rate when sulfur oxidizing microbes are present.

Dissolution kinetics is also affected by galvanic effects between various metal sulfides. The rate of sphalerite, galena and chalcopyrite dissolution was found to be slower in isolation. When leached in combination with pyrite, the rate of dissolution was 1.5, 31 and 18 times more rapid for the respective minerals (Abraitis *et al.* 2004).

In conclusion, various metal sulfides have been proposed to undergo oxidative mineral dissolution via different mechanisms depending on whether they are acid soluble or acid insoluble. This also results in the formation of varying end products. For chalcopyrite, these may result in the formation of precipitates on the mineral surface observed to hinder bioleaching (Klauber *et al.* 2003, Petersen *et al.* 2001). This is known as the *passivation of chalcopyrite* and may explain difficulties associated with incomplete leaching of chalcopyrite. High redox potentials also result in the formation of iron sulphate precipitates which impede chalcopyrite dissolution. Galvanic interactions between metal sulfides affect leach rates. Acid soluble minerals may result in the increase in pH over prolonged periods of bioleaching. The microbial consortia involved are therefore important in the regeneration of acid and ferric ions, and possibly in the removal of sulfur precipitates on the mineral surface, required for the dissolution to continue.

2.3.1.2 Effect of gangue mineralogy on bioleaching

In heap bioleaching, the ore is of a very low-grade such that the content of the mineral of interest is often less than 1 % in comparison to gangue (unwanted mineral). Gangue mineralogy and leach residues are being used increasingly as a means to predict heap leach efficiency (Helle *et al.* 2005) and economics as acid consumption by acid soluble gangue is a major cost factor (Watling 2006) in heap leaching. The formation of solids precipitated by the dissolution of gangue minerals has also been observed to be detrimental to overall bioleach efficiency (Watling 2006). Gangue mineralogy is specific to the mining region. However, predominating gangue minerals are usually quartz, the silicate orthoclase, feld spars such as plagioclase and the mica biotite. The nature and rate of dissolution of constituent gangue minerals is largely dependent on the nature of the primary rock forming minerals (Wilson 2004). However, dislocation and defect structures, mineral structure and chemistry, as well as the chemistry of the external solution, also contribute to the overall rate of dissolution observed (Wilson 2004). Minerals that crystallize at lower temperatures are more stable. Quartz, muscovite, K-feldspar (orthoclase) are therefore particularly resistant (Wilson 2004) to acid dissolution. The degree of exsolution[3] of feldspars may affect dissolution rates (Oxburgh *et al.* 1994).

2.3.2 Techniques available for mineralogical analyses

Current techniques for the quantitative analysis of mineral composition and leach residues include: X-ray Fluorescence (XRF), X-ray Diffraction (XRD) and QEMSCAN. Ore microscopy is an essential tool for the characterisation of minerals present; however, this technique provides only a qualitative assessment. These techniques, in combination, have been successfully used in combination for the characterisation of ore and leach residues and are discussed in the Chapter 3.

[3] Exsolution refers to 'the process whereby an initially homogeneous solid solution separates into two or more distinct crystalline phases without the addition or removal to or from the system' (http://www.eas.slu.edu/People/JPEncarnacion/mineralogy/week6.htm, 03-06-2007,15.00)

2.4. HOW DO MICROBES ADHERE TO SURFACES?

It is well established that certain microorganisms possess the ability to attach to solid surfaces (Watling 2006) with these surfaces being the major site of microbial activity (van Loosdrecht *et al.* 1990). In many cases, the association of microorganisms with a surface leads to the formation of a biofilm which the prevailing form of microbial life (Watnick and Kolter 2000). The phenomenon has been studied extensively within marine/aquatic and medical contexts (Bhinu 2005). Despite the rapidly expanding knowledge on microbial attachment and biofilm formation, there are still gaps in the understanding of this phenomenon in a bioleach environment. This section includes a brief introduction to adhesion of microbes to solid surfaces in general and with respect to bioleach microorganisms. The four stages of adhesion and colonisation are discussed in Section 2.4.1. This includes a discussion on the role of chemotaxis in primary attachment and the role EPS in the firm attachment, colonisation and the bioleach process. The factors influencing attachment in a bioleach environment are discussed in Section 2.4.2 and finally the prevalence of mineral selective attachment is discussed in Section 2.4.3.

2.4.1 The mechanism of attachment of microorganisms to solid surfaces

Attachment can be explained using second order irreversible rate kinetics with respect to bacterial concentration and substrate surface area in the system as proposed by Zobell (1943). The model comprises of two stages: an initial stage of reversible adhesion and a second irreversible attachment stage. van Loosdrecht *et al.* (1990), who reviewed the influences of interfaces on microbial activity, refined this model and proposed that attachment consists of a 4-stage sequence of events. Transport of the cell to the solid surface is followed by primary or initial attachment (Section 2.4.1.1). The next stage is known as firm attachment (Section 2.4.1.2) with the final stage being colonisation (Section 2.4.1.3). The model proposed by Zobell (1943), is used in combination with the attachment mechanism proposed by van Loosdrecht *et al.* (1990), to develop the most suitable representation of the adhesion process.

2.4.1.1 Primary attachment

The first step of bacterial adhesion requires the transport of the microbial cell to a surface in order for contact to be made (van Loosdrecht *et al.* 1990). Three modes of transport bring the cell in close proximity to the surface, namely: **diffusive transport, convective transport** and **active movement.**

Diffusive transport involves 'non-negligible Brownian motion'. This type of transport accounts for random contacts of small bacteria with surfaces. It facilitates crossing of the diffusive layer by the microorganism, which can not occur through convective transport alone. This process is slower than convective and active transport. **Convective transport** is due to liquid flow and may be several orders of magnitude faster than diffusive transport. However, there may be circumstances where the final path to the surface is still diffusion controlled. **Active transport** occurs in motile organisms. According to Watnick and Kolter (2000), the initial microbial-surface interaction is accelerated by force generating organelles such as flagella, responsible for active transport. *L. ferrooxidans* and *A. ferrooxidans* are both motile microorganisms possessing of flagella (Jerez 2001).

Once the organism is in the vicinity of the surface, the next stage of adhesion can take place in which the cells become associated with the surface either via a chemotactic response or by chance. This association is known as initial or primary adhesion; it is non-specific and may be reversible. Reversible adhesion will be defined as ''deposition to a surface in which bacteria can still be removed from the surface by mild shear or by the bacterium's own mobility'' (van Loosdrecht *et al.* 1990). Conversely, adhering bacteria which exhibit no Brownian motion and can only be removed by intense shear will be defined as being ''irreversibly attached''.

According to Zobell's model, the initial interaction between bacteria and surface sites requires the formation of a metastable complex between the two that is brought about by the balance of repulsive and attractive forces. This is represented in Equation 5, where, B represents bacteria, S represents surface sites involved, [BS]* represents the reversible attachment and formation of the metastable complex and BS represents irreversible attachment (adapted from Rodriguez *et al.* 2003**a**).

$$B + S \underset{K2}{\overset{K1}{\longleftrightarrow}} [BS]^* \xrightarrow{K3} BS \qquad \text{Eqn (5)}$$

The repulsive and attractive forces governing initial adhesion of microorganisms to surfaces are primarily due to physicochemical processes explained by non-specific hydrophobic and electrostatic interactions between the cell surface and minerals (van Loosdrecht *et al.* 1990, Devasia *et al.* 1993, Arrendondo *et al.* 1994, Blake *et al.* 1994 and Harneit *et al.* 2005), discussed in Section 2.4.2.

The association of microbial cells with a solid surface may occur as a chemotactic response or by chance. Chemotaxis refers to the response of microorganisms to a particular stimulus. The stimulus may be an 'attractant' such as a nutrient concentration gradient; in this case, motile bacteria swim towards higher concentrations of the stimulant. Conversely, the stimulus may be a noxious chemical (repellent), in which case motile bacteria swim away from higher concentrations of the stimulus. The process makes use of signal transduction proteins containing two functional domains: a sensory domain, which interacts with the stimulant at the cell surface; and a signalling domain, which modulates effector activities within the cell causing movement toward or away from the stimulus (Surette *et al.* 1994, Delgado *et al.* 1995; in Rojas-Chapana *et al.* 1998). This phenomenon has been well documented in *E.coli* and *Salmonella spp*. (Rojas-Chapana *et al.* 1998). However, little is known about the occurrence of chemotaxis in bioleach microorganisms. Rojas-Chapana *et al.* (1998) proposed the attachment of *A. thiooxidans* and *A. ferrooxidans* to sulfur/sulfide substrates as well as pyrite was due to chemotactic behaviour as this response was suppressed by addition of the detergent Tween 80. However, no evidence of the involvement of signal transduction proteins, the underlying mechanism of chemotaxis, was demonstrated.

Jerez (2001) isolated and characterised a gene from *L.ferrooxidans*, encoding a putative chemotactic receptor (LcrI). The LcrI protein structure was similar to that of methyl-accepting chemotaxis proteins, which suggests that *L. ferrooxidans* possesses a chemotactic signal transduction mechanism similar to that of other microorganisms. The association of bioleach microbes with the surface for the onset of initial adhesion may therefore be a result of a chemotactic response. However, more knowledge is required, with respect to the role of chemotaxis in adhesion of bioleach microbes to mineral surfaces.

2.4.1.2 Firm attachment

Firm attachment is the next stage of the attachment process and involves special cell surface proteins and or the excretion of polymers, which form strong links between the cell and surface (van Loosdrecht *et al.* 1990).

Devasia *et al.* (1993) suggested the involvement of a proteinaceous cell surface appendage in adhesion of *A. ferrooxidans* to solid mineral surfaces of sulfur grown cells. More recent reports of the involvement of cell-surface proteins in the attachment of bioleach microbes to mineral surfaces were made by Arrendondo *et al.* (1994). It was suggested that partial removal of lipopolysaccharides exposed greater levels of cell surface proteins, which may increase cell hydrophobicity resulting in increased levels of adhesion. This group also reported that phosphate starved cells displayed a greater cell surface protein content and exhibited increased levels of adhesion to sulfur prills, presumably due to increased hydrophobicity (Amaro *et al.* 1993, in Arrendondo *et al.* 1994). The involvement of cell-surface proteins in attachment in these instances is restricted to hydrophobic interactions involved in initial attachment. However, the involvement of cell surface proteins in attachment can not be completely ruled out as cell surface proteins have been shown to play an integral role in the biofilm formation of two extensively studied microorganisms, namely *E. coli spp*. and *Pseudomonas spp.* (Pratt and Kolter 1998, O'Toole *et al.* 1998 respectively). Blake *et al.*

(2001) propose a model involving the cell surface protein, aporusticyanin, in the adhesion of *A. ferrooxidans*. According to this model aporusticyanin acts a mineral-specific receptor for the initial adhesion of *A. ferrooxidans* to pyrite (Blake *et al.* 2001).The role of excreted polymers in attachment is discussed in Section 2.4.1.3.

2.4.1.3 The role of extra-cellular polymeric substances (EPS) in the attachment process

As early as 1943, the presence of an excreted mucilaginous layer has been observed to enhance microbial attachment to surfaces. This was observed by Zobell (1943). This mucilaginous layer is in fact a result of the excretion of lipopolysaccharides or extra cellular polymeric substances (EPS) (Purevdorj-Gage and Stoodley 2004, p164) and mediates attachment and biofilm formation in bioleach microbes (Savage and Fletcher 1985, in Gehrke *et al.* 1998). EPS excretion is thus important in both the firm attachment and colonization stages of attachment.

Vandevivere and Kirchman (1993) report that the presence of solid surfaces may stimulate bacteria to produce exopolymers, as the addition of sand to shake flask cultures induced exopolymer synthesis. In addition, exopolymer production by attached cells was greater than planktonic cells and when surface-grown cells were resuspended in fresh medium, exopolymer production decreased to that indicative of planktonic or unattached cells. Due to the enhanced production of exopolymers in the presence of a solid substrate it could be inferred that the production of exopolymers indeed plays a significant role in attachment. (The exact nature of this putative effect of solid surfaces on exopolymer synthesis is not known.)

Gehrke *et al.* (2001) report that growth of *A. ferrooxidans* on different substrata influenced the yield of EPS produced. Cells grown on ferrous sulphate produced less EPS than cells grown on a solid substrate (pyrite). This supports the claims of Vandevivere and Kirchman (1993). However, if these claims are indeed significant then the presence of solid substrata alone would enhance EPS production. The substrata used by Gehrke *et al.* (2001) provide a source of energy for the microorganism, and thus for the production of EPS. Attachment to the material would be advantageous for survival of the organism.

Arrendondo *et al.* (1994) demonstrated the involvement of excreted lipopolysaccharides in the attachment of *A. ferrooxidans* to sulfur prills, as partial removal of lipopolysaccharides diminished the attachment observed. In another study, cells containing EPS attached to covellite surfaces whereas cells without EPS where unable to attach until EPS was regenerated (Pogliani and Donati 2000).

Escobar *et al.* (1996) investigated the attachment of *A. ferrooxidans* to pyrite and chalcopyrite in shake flasks (cells enumerated using direct cell counts). Cells were treated with EDTA to remove lipopolysaccharides from the cell surface. Loss of lipopolysacharides of the outer membrane in *A. ferrooxidans* negatively influenced attachment. Therefore, it was presumed that the presence of lipopolysaccharides play a role in the adsorption of this microorganism to solid surfaces, in accordance with the findings of Arrendondo *et al.* (1994).

Gehrke *et al.* (1998) also demonstrated that EPS excretion is necessary for the attachment of *A. ferrooxidans* on pyrite and sulfur solid surfaces and proposed that the complexation of ferric ions with glucuronic residues within EPS contributes to electrochemical interactions between the cell and mineral surfaces. In 2001, this group proposed that since charge effects are responsible for initial adsorption interactions, EPS-complexed iron species (Fe^{3+}) would be attracted preferentially to the negatively charged surface of pyrite (at pH 2.0) as EPS-complexed iron serve as Lewis acids (accepting unshared electron pair of pyretic sulfur). The group goes on the report that attachment to hydrophobic substrata such as sulfur would be dominated by van der Waal's attraction forces, and suggests that pyrite-grown cells possess an intermediate chemical EPS-composition which may enable attachment to both hydrophobic and charged surfaces. In addition, Gehrke *et al.* (1998 and 2001) analysed the chemical composition of partially purified EPS. EPS excreted by *A. ferrooxidans* was comprised predominantly of neutral sugars and lipids with the composition dependent on growth medium (discussed in Section 2.4.2). Similar findings were observed by Kinzler *et al.* (2003) and Harneit *et al.* (2005). Harneit *et al.* (2005) demonstrated that attachment of *A. ferrooxidans* was diminished when the cells were depleted of EPS. The role of EPS in bioleaching is discussed in Section 2.1.

2.4.1.4 Microbial colonisation of solid surfaces

Following initial attachment, which is a transient, reversible stage of attachment (van Loosdrecht *et al.* 1990), metabolic processes such as the production of EPS may enhance the extent of subsequent microbial adhesion (Yee *et al.* 2000) resulting in the establishment of a more firm attachment. This leads to the final stage of attachment, namely surface colonisation. Cells become embedded in EPS and firmly attached cells begin to grow and multiply, while remaining attached to the surface and each other (Watnick and Kolter 2000). Also, new planktonic cells may become firmly attached (Watnick and Kolter 2000). This leads to the development of microcolonies or biofilms. A biofilm may be described as a complex sessile community of microorganisms, which is highly differentiated and enveloped in a polysaccharide matrix. Colonisation and biofilm formation may be dependent on the organism type involved and these processes are therefore more specific. The exact nature of this matrix may vary depending on surrounding environment and the micro-organisms involved (Harneit *et al.* 2005). Ohmura *et al.* (1993) report that low coverage ratios of *A. ferrooxidans* to pyrite may be indicative of cells adsorbing as a single layer. Harneit *et al.* (2005) report the development of micro-colonies, covered in EPS, on the surface of pyrite by *A. ferrooxidans.* The micro-colonies subsequently developed into mono-layered biofilms as observed using atomic force microscopy.

Biofilm formation has been shown to be coordinated by diffusible signal transduction mechanisms, such as quorum sensing in *Pseudomonas* and *Vibrio fisherii.* Farah *et al.* (2005) have recently isolated genes which may encode for a functional type AI-1 quorum sensing system in *A.ferrooxidans*. The advantages of attachment and biofilm formation are discussed in Section 2.6.

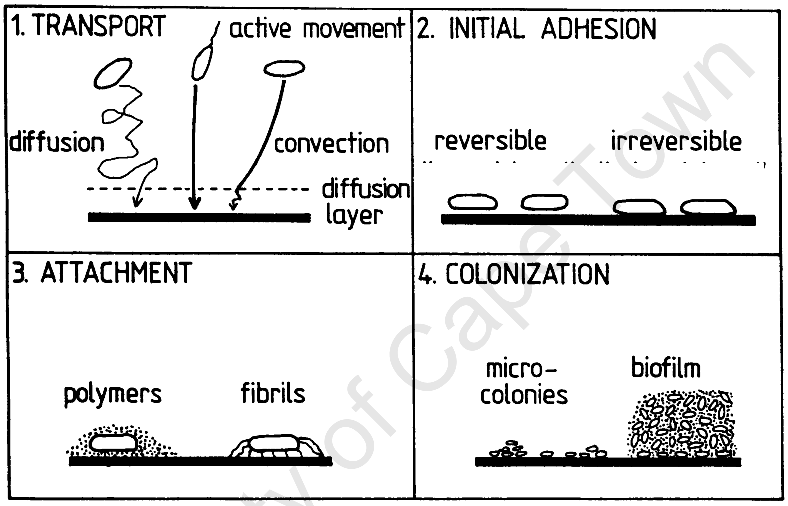

Figure 2.2: Four stages involved in attachment of microorganisms to solid surfaces, taken from van Loosdrecht *et al.* (1990).

2.4.2 The influence of surface chemistry in the adhesion mechanism: Extended Derjaguin, Landau, Verwey, Overbeek (XDLVO) theory and electric double layer interactions

Initial or primary microbial adhesion to solid substrata, as discussed in Section 2.4.1, is due to transient, non-specific electrostatic and hydrophobic interactions between the two surfaces involved. The interactions between microorganisms and mineral surfaces responsible for initial adhesion have been described using colloidal chemical theories such as the Extended Derjaguin-Landau-Verwey-Overbeek (XDLVO) theory. This theory accounts for electric double layer and Van der Waals forces between two charged surfaces, which together account for electrostatic interactions, and make use of the change in Gibbs energy as a function of distance between the two bodies.

The Gibbs energy is obtained from the sum of the Van der Waals and electric double layer interactions, provided steric effects are not significant (van Loosdrecht *et al.* 1990). The Lifshitz van der Waals interactions are weak, attractive, long range interactions (involving forces between permanent dipoles-permanent dipoles, permanent dipole-induced dipole, and induced dipole-induced dipole interactions); with electric double layer forces being stronger, long range, repulsive interactions (as both bacterial and mineral surfaces submerged in aqueous environments are usually negatively charged).

As previously mentioned, solid surfaces submerged in an aqueous medium are almost always electrically charged. This is predominantly due to the ionization of surface molecular groups and the adsorption of ions contained in the solution onto the surface of the submerged solid (Blake *et al.* 1994). Counterions (ions carrying opposite charge to substrata) are attracted to the solid surface, and co-ions (like charged ions) repelled. The surface charge of the submerged solid, along with the charge imposed by excess adsorbed counterions, form part of the electrical double layer (Blake *et al.* 1994). The electrical double layer consists of a stern layer, where counterions are tightly associated with the solid surface and neutralise most of the surface charge, and a diffuse layer, where the remaining surface charge is balanced by counterions – the concentration of which decreases with distance from the solid surface (Blake *et al.* 1994). The electrical double layer is shown schematically in Figure 2.3, where the stern and diffuse layers can be seen. At low ionic strength, the electric potential decreases exponentially with distance from the charged surface, seen in diagram (B) of Figure 2.3 (van Loosdrecht *et al.* 1990, Blake *et al.* 1994, Poortinga *et al.* 2002).

The thickness of the diffuse layer is affected by ionic strength and by the pH of the suspending solution and influences the electrostatic interactions between the microbial and solid surface. Increasing ionic strength decreases the diffuse layer thickness resulting in a decrease in the range of electrostatic repulsion (van Loosdrecht *et al.* 1990, Blake *et al.* 1994, Poortinga *et al.* 2002).

In conclusion, interactions between the electrical double layers of two surfaces contribute to electrostatic interactions in aqueous environments. These interactions are usually repulsive as most microbial and mineral surfaces are negatively charged in water, and are long range interactions (Poortinga *et al.* 2002, Ohmura *et al.* 1993, Blake *et al.* 1994, van Loosdrecht *et al.* 1987a) in low ionic strength solutions. The pH and ionic strength of the suspending solution influence electrostatic interactions by affecting the thickness of the diffuse layer. In addition to electrostatic interactions, XDLVO theory accounts for short range Lewis acid-base or hydration interactions (Poortinga *et al.* 2002). When hydrophobic interactions are substantial, adhesion is said to be spontaneous (ΔG adhesion is negative) (Nagaoka *et al.* 1999) and electrostatic interactions may play a less integral role in the adhesion interactions between two surfaces under these conditions (Rijnaarts *et al.* 1993). Hydrophobicity is thus also an important contributing factor to the initial adhesion interactions. XDLVO theory has been effectively applied to describe bacterial adhesion (Poortinga *et al.* 2002).

The concepts of electrostatic and hydrophobic interactions in aqueous environments, as described by Extended Derjaguin, Landau, Verwey, Overbeek (XDLVO) theory is used to understand initial adhesion interactions of microorganisms to mineral surfaces.

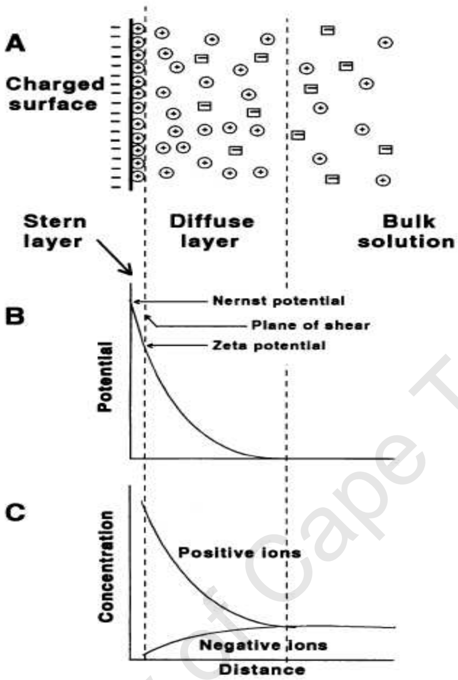

Figure 2.3: Schematic representation of the electrical double layer (A) possible structure of the solid-electrolyte interface. Anions represented by negative signs and cations by positive signs. (B) Dependence of electrical potential on distance from charged surface. (C) Dependence of the concentration of cations and anions in the electrolyte on the distance from the negatively charged surface. (Sourced from Blake *et al.* 1994).

2.4.3 The influence of microbial surface properties on initial adhesion interactions

A brief discussion is presented with regard to the main cell surface components contributing in the Gram negative bacteria to surface charge and hydrophobicity, as these are important factors to be considered when making inferences about initial adhesion interactions.

2.4.3.1 The microbial cell wall

Microbial surfaces are usually negatively charged under physiological conditions (Poortinga *et al.* 2002, Blake *et al.* 1994, van Loosdrecht *et al.* 1990, Ohmura *et al.* 1993). However, this varies from species to species. Cell surface charge arises due to dissociation or protonation of the carboxyl (-COOH), phosphate, hydroxyl (-OH) and amino groups ($-NH_2$) which constitute the cell wall. This dissociation and protonation is dependent on pH (Sampson *et al.* 2000, Poortinga *et al.* 2002, Sharma *et al.* 2003). Various components of the microbial cell wall contribute to the overall surface charge and hydrophobicity. The charge and hydrophobicity of these components may be affected by growth conditions and solution chemistry. The mesophilic microorganisms used in this research are Gram negative. The components of Gram negative bacteria include a peptidoglycan layer (1 - 2 nm thick), flanked by phospholipids bilayer membranes, namely the cytoplasmic and outer membranes, separated by a periplasmic space as depicted in Figure 2.4.

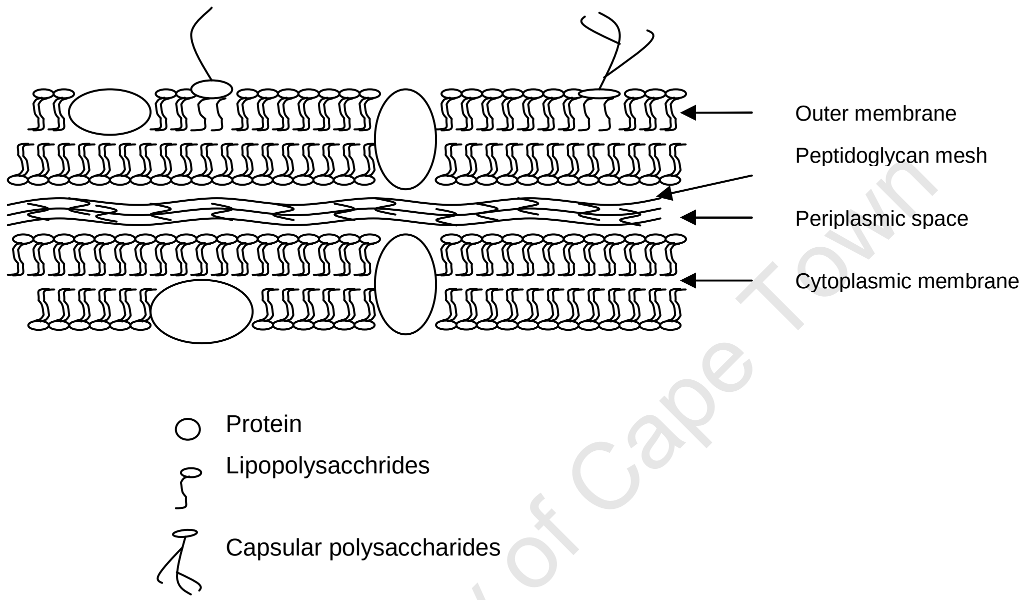

Figure 2.4: Schematic representation of Gram negative cell wall components.

The outer membrane contains membrane associated proteins which have been reported to contribute to surface charge (Poortinga *et al.* 2002) and hydrophobicity (van Loosdrecht *et al.* 1987, Arrendondo *et al.* 1994). The outer membrane of Gram negative bacteria may also contain lipopolysacchride chains which confers a negative charge and is hydrophilic in nature. Some Gram negative cell envelopes also contain a capsule or S-layer linked to the lipopolysaccharide layer. This S-layer may vary in thickness, is usually anionic and may render the cell surface hydrophobic by partially masking the hydrophilic lipopolyssacharide surface (Sleytr *et al.* 2001). Many bacterial species produce EPS which contributes to cell surface charge and hydrophobicity, the role of EPS in attachment has been discussed in Section 2.4.1.3.

2.4.3.2 Electrokinetic potential of microbial surfaces

Electrostatic interactions have been shown to play a significant role at low ionic strength and when hydrophobicity is not a large contributing force (Rijnaarts *et al.* 1993). Determining the electrical potential via zeta-potential measurements at each surface allows quantitative means of obtaining information on surface charge and thus electrostatic interactions (van Loosdrecht *et al.* 1987**a**). The surface charge, measured using zeta-potential measurements as cited in literature, for microorganisms pertaining to this study is presented and discussed in this section.

Devasia *et al.* (1993) measured the surface charge of *A. ferrooxidans* grown on various growth substrates, namely pyrite, chalcopyrite, sulfur and ferrous sulphate. They report an isoelectric point at (approximately) pH 2.0 for ferrous grown cells, with the surface charge becoming increasingly negative as pH increased. However, cells grown on mineral substrates (sulfur, pyrite and chalcopyrite) exhibited an isoelectric point at approximately pH 3.8. Thus the presence of the solid growth substrata affected cell surface charge. It is also reported that surface coverage of solid substrates is at a maximum density slightly above the isoelectric point of the adhering cells i.e. where cells are slightly negatively charged (Mozes and Rouxhet in Devasia *et al.* 1993).

Ohmura *et al*. (1993), report a maximum negative surface charge of -4.6 mV for ferrous sulphate grown *A. ferrooxidans* (cultured at pH 2.5) measured in a sulfuric acid solution at pH 2.0. This is inconsistent with reports made by Devasia *et al.* (1993) who report *A. ferrooxidans* to exhibit an isoelectric point at this pH.

Blake *et al.* (1994), observe a dependence of surface charge on the concentration of ferric ions and growth stage of the *A. ferrooxidans* culture. *A. ferrooxidans* cultured on ferrous sulphate was harvested from a stationary phase environment which was saturated with ferric ions. The zeta-potential measured at pH 2.0 for these cells ranged between -5.0 mV and +1.5 mV and varied from culture to culture. These values are within range of reports made by Devasia *et al.* (1993) and Ohmura *et al.* (1993). Surface charges of *A. ferrooxidans* in the absence of ferric ions varied from -7.4 mV to -1.8 mV. Upon addition of ferric ions, the surface charge (zeta potential) of *A. ferrooxidans* increases to a maximum positive value of 2.1 mV. It was suggested that ferric ions adsorb preferentially to *A. ferrooxidans* in the stern layer, which neutralises the negative charge of the cell surface.

Sharma *et al.* (2003) measured the surface charge for ferrous sulphate, sulfur and pyrite grown *A. ferrooxidans* at pH 2.0 at an ionic strength of 10^{-3} M KCl. Ferrous grown cells exhibited an isoelectric point at a pH approximately less than pH 2.0, above which the surface charge became increasingly negative. A maximum negative surface charge of -10 mV was reported for ferrous grown *A. ferrooxidans* measured at pH 7. The surface charge of ferrous grown *A. ferrooxidans* at pH 2.0 reported by these authors is within the range reported by Devasia *et al.* (1993). An isoelectric point was observed between pH 3.0 and pH 3.5 for both sulfur and pyrite grown cells, below which the surface charge was positive. At pH 2.0 a positive surface charge was reported for both pyrite and sulfur grown cells. Above the isoelectric point, pyrite grown cells became increasingly negative and exhibited a maximum negative charge of -25 mV at pH 7. Pyrite grown cells exhibited a more negative surface charge than cells cultured on ferrous sulphate. This observation, together with isoelectric points and surface charges reported for *A. ferrooxidans* cultured on mineral substrata is in accordance with claims made by Devasia *et al.* (1993).

4.2.3.3 Hydrophobicity of microbial surfaces

Van Loosdrecht *et al.* (1987**b**) found that surface hydrophobicity was the more influential parameter to be considered, as complete adhesion was observed for microorganisms with high hydrophobicities (having high contact angles) irrespective of electrophoretic mobility (charge). Contrastingly, the electrokinetic potential became more influential for hydrophilic cells. In addition, the authors report high negative electrokinetic potentials for hydrophobic cells. It was suggested that although this combination may conflict with the established theory, the charged groups occupy a minor proportion of the total surface area. Thus hydrophobicity may play a significant role and may be regarded as the thermodynamic driving force of initial adhesion, under conditions where both mineral and microbial surfaces are highly hydrophobic.

Rijnaartss *et al.* (1993) also observed the significance of hydrophobicity over electrokinetic potential in the initial adhesion of microorganisms to Teflon and glass surfaces. Cells exhibiting greater hydrophobicity adhered more readily to surfaces which were more hydrophobic. It was suggested that hydrophobicity of the two surfaces yielded greater attractive forces and thus higher levels of adhesion, whereas electrokinetic potentials of the surfaces involved only had a significant effect on adhesion when hydrophobicity was negligible and at lower ionic strength solutions. Under these conditions the electrostatic interactions are more repulsive and lower levels of adhesion are observed. This is in agreement with the findings of van Loosdrecht *et al.* (1987**b**).

Hydrophobicity measurements using contact angles and biphasic partitioning systems have been used in the understanding of primary adhesion (van Loosdrecht et al. 1987**b**, Ohmura et al. 1993, Devasia et al., 1993).

Porro *et al.* (1997) found an increase in cell surface hydrophobicity of sulfur grown *A. ferrooxidans* measured using biphasic liquid-liquid partitioning. It was reported that this increase in hydrophobicity led to increased attachment to substrata which are also hydrophobic. Similarly, Devasia *et al.* (1993) reported increased hydrophobicity for *A. ferrooxidans* cells cultured on solid substrates (sulfur, pyrite and chalcopyrite) relative to ferrous sulfate cultured cells using liquid-liquid partitioning in an aqueous and organic phase (*n* – hexane) as well as affinity chromatography using octyl-Sepharose (hydrophobic cells will have a greater affinity for octyl-Sepharose). Higher levels (20%) of mineral cultured cells were partitioned to the hydrophobic *n* – hexane phase relative to ferrous sulphate grown cells. In addition, greater proportions (over 50%) of mineral grown cells remained bound to the hydrophobic octyl-Sepharose where as the proportion of cells cultured on ferrous sulphate bound to the octyl-Sepharose was substantially lower (20%).

Solari *et al.* (1992) demonstrated an increase in bacterial hydrophobicity with the decrease in solution pH which affected attachment efficiency with bacteria adhering more readily to hydrophobic surfaces (sulfide minerals) under these conditions.

Ohmura et al. (1993), observed that *E. coli* was more hydrophobic than *A. ferrooxidans* using contact angle measurements and biphasic systems. The *A. ferrooxidans* contact angle measured in sulfuric acid on glass was 22.7° and on acrylic surfaces was 24.0°

2.4.4 The influence of mineral surface properties on initial adhesion interactions

Surface charge and hydrophobicity, surface tension, and mineral surface roughness have been suggested as factors which control the initial adhesion of microorganisms to mineral surfaces (Yee *et al.* 2000). These mineral properties are discussed.

2.4.4.1 Mineral surface electrokinetic potential and hydrophobicity

The surfaces of sulfide minerals are usually negatively charged (Ohmura *et al.* 1993). For sulfide minerals under investigation, charge is determined via the oxidation state of the mineral. In this study, quartz is used as a control mineral. The potential determining ions of quartz (SiO_2) are the - OH groups present. The surface charge of oxides (quartz) is thus affected by the pH of the suspending solution. As the pH decreases, the surface becomes more positively charged. Escobar *et al.* (1996) report that quartz exhibits an isoelectric point at pH 2.0 above which it is negatively charged. Yee *et al.* (2002) model surface species of quartz and predict the isoelectric point of quartz occurs at approximately pH 3.0. Again, measurement of contact angles can be used as an indication of the relative hydrophobicity of the mineral surface. The surface charge and contact angles of the minerals of interest are summarised in Table 2.2.

Table 2.2: For minerals of interest the surface charge and hydrophobicities are presented. Where surface charge was not available, the isoelectric point (IEP) and corresponding pH is cited.

Author	**Mineral**	**ζ (mV) at pH 2.0**	**Contact angle θ at pH 2.0**
Ohmura *et al.* (1993)	Pyrite	- 7	68.9 ± 2.1
	Chalcopyrite	-	83.4 ± 4.5
	Quartz	-	28.4 ± 4.3
Nagaoka *et al.* (1999)	Pyrite	- 28.12 (± 9.96)	83.8 ±1.3
Devasia *et al.* (1993)	Pyrite	IEP pH 2.9	*
	Chalcopyrite	IEP pH 2.6	*
Yee *et al.* (2002)	Quartz	IEP pH 3.0	*
Kitchner J. A. (1992)	Quartz	IEP pH 2.0	*
Escobar *et al.* (1996)	Quartz	IEP pH 2.0	*

The minerals used in this experiment decrease in surface hydrophobicity in the following order: chalcopyrite, pyrite, quartz, measured using contact angles (Ohmura *et al.* 1993). However, the hydrophobicity of pyrite, as per contact angle, reported by Nagaoka *et al.* (1999) was similar to that reported for chalcopyrite by Ohmura *et al.* (1993); thus these two minerals may be equally hydrophobic. The hydrophobicity reported for quartz was substantially lower than for the sulfide minerals; thus this was the least hydrophobic or most hydrophilic component used.

2.4.4.2 Physical properties of mineral surfaces

Certain bioleach microbes have displayed ‘affinities’ for attachment to visible defects and particular mineral properties. Gehrke *et al.* (1998) observed, using atomic force microscopy (AFM), that *A. ferrooxidans* attached to dislocation sites (cracks and grain boundaries) on pyrite. Some 76 % of visible cells adhered to these surface imperfections. Similar reports were made by these authors in 2001, where again using AFM, *A. ferrooxidans* was observed to be attached, specifically to dislocation sites on pyrite surfaces. Andrews (1988), in Gehrke *et al.* (1998 and 2001), suggests that sulfur atoms concentrate at grain boundaries and surface imperfections making these locations more favourable as sites of attachment. Rojas-Chapana *et al.* (1998) also showed increased attachment at pyrite grain boundaries. Nutrient concentration gradients were proposed to be generated at pyrite fragmentation sites, which attracted the settlement of microbes at these specific sites over others. Harneit *et al.* (2005) also observed that primary attachment was restricted to surface sites with visible defects.

Sanhueza *et al.* (1999) correlated the degree of crystallization of synthetic pyrite films to bacterial attachment behaviour. Amorphous pyrite exhibited elongated densely packed arrangements of adhered *A. ferrooxidans.* However, on highly crystallized pyrite, short, pearl-like chains where observed with a lower overall surface area coverage. Gonzalez *et al.* (1999) showed that attachment levels decrease with mineral particle size, due to the decrease in available surface area.

2.4.5 The effect of varying microbial growth conditions on cell surface properties and attachment

A liquid medium that is rich in nutrients primes many bacteria for attachment to any local surface (Prigent-Combaret *et al.* 1999, in Watnick and Kolter 2000, O'Toole *et al.* 1998 and Pratt and Kolter 1998). In addition to this, surface chemistry determining adhesion is influenced by growth conditions (van Loosdrecht *et al.* 1987**b**). Gehrke *et al.* (1998 and 2001) correlated the influence of growth history of *A. ferrooxidans* to changes in cell surface properties, EPS yield and composition, and subsequent attachment ability. Growth on solid pyrite enhanced the yield of EPS relative to ferrous grown cells. This in turn altered the cell surface properties as the complexation of ferric ions with gluco-uronic residues in the EPS layer rendered the cell surface positively charged allowing microorganisms to adhere to both hydrophobic and negatively charged surfaces. Similarly, Harneit *et al.* (2005) observed this dependence of EPS composition on growth medium and subsequent attachment ability imposed on cells.

Amaro *et al.* (1993, in Arrendondo *et al.* 1994), also observed that attachment could be affected by growth conditions. This group demonstrated that phosphate starved *A.ferrooxidans* cells have greater cell surface protein content and display increased degrees of hydrophobicity, which affected attachment behaviour. Arrendondo *et. al.* (1994) also reported that EPS production enhances attachment levels.

Porro *et al.* (1997) investigated the relationship between growth conditions, cell surface hydrophobicity and adhesion behaviour. *A. ferrooxidans* cells were grown on sulfur or on ferrous iron and then subsequently grown on wurtzite. Sulfur grown cells were most hydrophobic (using bi-phasic partitioning). However, greater attachment efficiencies were achieved with mineral grown cells over ferrous grown cells, despite these cells having hydrophobicities.

Sampson *et al.* (2000) observed a correlation between growth history, cell surface hydrophobicity and subsequent adhesion behaviour using the theoretical energy of adhesion of cells grown on the various substrates (sulfur, chalcopyrite and ferrous iron). Sulfur grown cells were predicted to have the greatest free energy of adhesion and thus should be more likely to adhere to hydrophobic surfaces.

Devasia *et al.* (1993) observed that sulfur, pyrite and chalcopyrite grown *A. ferrooxidans* exhibit greater hydrophobicity than ferrous iron grown cells. In addition, cells cultured on solid media exhibited an isoelectric point at a higher pH than ferrous grown cells. The growth substrate therefore also affected surface charge of the microorganism. Sharma *et al.* (2003) characterised *A.ferrooxidans* cell surface groups using diffuse and reflectance FT-IR and FT-Raman spectroscopies. Cells were cultured with ferrous ion, sulfur or pyrite mineral as sole energy source. The cell surface charge of iron-grown cells (isoelectric point at pH 2.0) was different to that of cells grown on solid substrata (pyrite) (isoelectric point pH 3.0 – 3.5). Therefore, surface charge was dependent on growth medium of the microorganism. Similarly, Blake *et al.* (1994) observed a dependence of surface charge on the growth history of the *A. ferrooxidans* cultured on ferrous sulphate, pyrite and sulfur.

2.4.6 The effect of microbial growth phase on adhesion behaviour

Several other authors have noted the influence of the activity of the microorganism on the attachment efficiencies observed (Zobell 1943, van Loosdrecht *et al.*1990 and Rijnaarts *et al.* 1993). Zobell (1943) observed that more cells attach during early logarithmic growth than during late log or lag phase i.e. when the activity of the cells was not optimum. van Loosdrecht *et al.* (1987) related the growth rate and activity level of microorganisms to attachment levels (higher dilution rates related to higher attachment levels). Similarly, Elwood *et al.* (1982, cited in van Loosdrecht *et al.*1990) and Rijnaarts *et al.* (1993) report that the adhesion of microbes may be increased during exponential growth. Elwood postulated this to be due to increased cell wall hydrophobicity during this phase.

2.4.7 The effect of solution chemistry on microbial attachment behaviour

2.4.7.1 The influence of solution pH on attachment of microorganisms to mineral surfaces

Primary adhesion is due to physicochemical processes explained by non-specific hydrophobic and electrostatic interactions between the cell surface and minerals. Bacterial cells are generally negatively charged and hydrophobic in nature; however, this varies from species to species. Electrostatic interactions, a result of the electrical double layer interactions between two surfaces, are affected by ionic strength and pH of the solution. The solution pH also determines speciation of cell wall components, determining charge (Yee *et al.*2000).

Solari *et al.* (1992) demonstrated an apparent increase in bacterial hydrophobicity with the decrease in solution pH. This affected the attachment and bacteria adhered more readily to hydrophobic surfaces (in this case sulfide minerals) under these conditions. Escobar *et al.* (1997) observed an increase in the attachment of *A. ferrooxidans* to glass beads as pH decreased. The authors claim that decreasing the pH enhanced production of lipopolysaccharides making the surface of the cells more hydrophobic. As the pH in decreased, the surface of the glass reached its isoeletric point (at about pH 2.0), therefore the effect oh hydrophobic interactions between cells and mineral surface became dominant with greater attachment.

2.4.7.2 The effect of solution ferrous:ferric ion ratio in solution on microbial adhesion

Ohmura *et al.* (1993) demonstrated that ferrous ion inhibited the attachment of *A.ferrooxidans* to pyrite and chalcopyrite. The presence of ferric ions was less inhibiting to attachment levels of *A. ferrooxidans* to the respective minerals. The ionic strength of the ferric ion solution was greater than the ionic strength of the ferrous ion solution under experimental conditions. Therefore, the effect of ferrous ion concentration on attachment of *A. ferrooxidans* was not a result of ionic strength of the solution. It was presumed that excess ferrous iron competitively inhibited the attachment of *A. ferrooxidans* pyrite and chalcopyrite, as *A. ferrooxidans* oxidizes ferrous iron as a source of energy.

Gonzalez *et al.* (1999) observed that cell attachment increased with the addition of ferric ions. The mineral surfaces involved in bioleaching are usually negatively charged. Ohmura et al. (1993) found the surfaces of pyrite, chalcopyrite and galena to be negatively charged by measuring zeta potentials. Blake *et al.* (1994) proposed that addition of ferric ions shifts the net charge of the cell surface asymptotically to a positive value of 2.1 mV at pH 2.0, owing to the ferric ions adsorbing preferentially to *A. ferrooxidans.* This explains the observations of Gonzalez *et al.* (1999). Ferric ion complexation by EPS is proposed by Gehrke *et al.* (1998). According to this theory, complexation of ferric ions through glucuronic acid residues of EPS gives the cell surface a positive charge, which contributes to electrochemical interactions between the cell and mineral surfaces and thus may increase attachment. Hansford and Vargas (2001) observed that redox potential and concentration of soluble iron in solution influences the ferric/ferrous ratios within the EPS, thereby affecting attachment behaviour.

2.4.8 The effect of shear on initial mineral-microbe interactions

Rijnaarts *et al.* (1993) report fluid velocities in batch flask attachment studies to influence adsorption observed. Under conditions of high shear, lower levels of attachment were observed for hydrophilic strain-surface combinations, with greater resistance to shear environments observed for hydrophobic strain-surfaces combinations. This study did not make use of bioleach microorganisms. However, it demonstrated that weak charge-based interactions may be compromised under conditions of high fluid velocity. Hydrophobic interactions result in greater attractive interactions offering some resistance to shear forces, and may play a more significant role in initial microbe-mineral interactions and subsequent deposition in flowing systems

2.4.9 Selective attachment of microorganisms to sulfide minerals

The location of attachment of the microbes to the mineral surface is expected to impact attachment and subsequent bioleaching performance. Several authors (Ohmura *et al.* 1993, Nagaoka *et al.* 1999, Gonzalez *et al.* 1999, Sampson *et al.* 2000, Rodriguez *et al.* 2003 and Harneit *et al.* 2005) have demonstrated the prevalence of selective attachment to sulfide minerals over other minerals.

Solari *et al.* (1992, in Watling 2006), observed increased levels of adhesion of *A. ferrooxidans* to sulfide minerals per unit area over quartz. This was attributed to hydrophobic interactions between cells and sulfide mineral surfaces, as both cell and sulfide mineral whereas quartz is more hydrophilic. Ohmura *et al.* (1993) demonstrated selective adhesion of *A. ferrooxidans* to sulfide minerals over other minerals in shake flask experiments using *E. coli* as a control organism. *A. ferrooxidans* was shown to attach preferentially to pyrite, followed by chalcopyrite, with little attachment to quartz and galena. The attachment was shown to be selective by using hydrophobicity as well as cell and mineral surface charge measurements. For selective attachment to occur, these forces must be overcome as both the cell and mineral surface have negative charges and display varying degrees of hydrophobicity. Attachment levels were shown to increase with an increase in cell concentration. This increase in the level of attachment continued until a certain threshold concentration was reached, beyond which no further increase in attachment could be seen. The maximum level of attachment could be due to spatial distribution of microbes due to repulsive forces of cell surfaces.

Similarly, Nagaoka *et al.* (1999) and Gonzalez *et al.* (1999) showed selective adhesion of *A. ferrooxidans* to pyrite over other minerals using a range of cell concentrations. Attachment increased with cell concentration, and in addition to this, an increase in adhesion with mineral (pyrite) concentration was also shown (Nagaoka *et al.* 1999). Gonzalez *et al.* (1999) showed that attachment occurs rapidly. The level of attachment achieved was influenced by the liquid environment. Maximum concentration of attached cells increased with the addition of ferric ions (see Section 2.4.7.2).

Sampson *et al.* (2000) demonstrated that non-specific hydrophobic interactions do not control attachment of the mesophile *A. ferrooxidans* which attaches selectively to arsenopyrite, followed by pyrite and chalcopyrite. Similarly, a similar trend of selective attachment of various thermophilic bioleach micro-organisms exhibited increased levels of attachment to pyrite, followed by arsenopyrite and chalcopyrite. These results are in agreement with Rodriguez *et al.* (2003**a**) who demonstrated selective attachment of mesophiles and thermophiles to sulfide minerals using mixed cultures. The Mesophiles, *Acidithiobacillus spp*. and *Leptospirillum spp*., exhibited higher levels of attachment to pyrite followed by chalcopyrite and sphalerite. The affinity of the thermophile, *Sulfolobus spp.*, differed with preferential attachment to chalcopyrite and sphalerite followed by pyrite. Higher overall attachment efficiencies were achieved by thermophiles. Presence of ferrous iron resulted in detachment, corroborating that the liquid environment influences the degree of attachment achieved (Gonzalez *et al.*1999). Supplying excess ferrous iron decreases adhesion of *A. ferrooxidans* to mineral surfaces as the chemotactic benefit is removed. The results for selective attachment of thermophiles contradict claims made by Sampson *et al.* (2000) who report preferential attachment of thermophiles to pyrite over chalcopyrite. The most recent study of Harneit *et al.* (2005) demonstrated rapid selective attachment of *A. ferrooxidans* to pyrite, with slower rates of attachment to chalcopyrite, sphalerite and galena. This is in agreement with Gonzalez *et al.* (1999) that attachment occurs rapidly; and with Rodriguez *et al.* (2003) that *A. ferrooxidans* attaches selectively to sulfide minerals.

The current body of literature provides evidence which suggests that bioleach microorganisms attach preferentially to sulfide minerals. This adhesion to sulfide minerals is selective as factors governing non-selective adhesion must be overcome for this attachment to occur. The attachment to the sulfide minerals is rapid and increases with cell concentration until a threshold concentration is attained; above this no further increase in attachment levels can be seen.

2.5 THE EFFECTS OF ATTACHMENT ON BIOLEACHING

The role of microbes in bioleaching via the indirect contact mechanism is to regenerate ferric iron and protons required for mineral dissolution (Section 2.1). Many microorganisms excrete EPS for biofilm formation and the creation of favourable microenvironments. Via the indirect mechanism, the excretion of EPS also plays a role in bioleaching as it brings the microorganisms and the reactants for mineral oxidation in close proximity to the mineral surface. Several authors have provided experimental evidence which correlates microbial attachment to enhanced dissolution rates of certain minerals in comparison to abiotic leaching as well as leaching with planktonic cells.

Gehrke *et al.* (1998) investigated the importance on EPS in bioleaching. The dissolution rates of pyrite by abiotic leaching, inactive bacteria (*A. ferrooxidans*) containing EPS, active bacteria with no EPS and active bacteria containing EPS were determined. Higher dissolution rates were observed for active cells containing EPS. Microbial attachment was therefore correlated to an enhancement of the overall dissolution rate. Fowler and Crundwell (1999) used scanning electron microscopy (SEM) to demonstrate that *A. ferrooxidans* attached to sphalerite mineral surfaces. The dissolution rates of sphalerite was measured in abiotic and biotic conditions, with higher dissolution rates of sphalerite observed with attached *A.ferrooxidans* compared to abiotic leaching. Rodriguez *et al.* (2003) assessed the degree of bacterial attachment and its influence on the mineral dissolution rate. A mixed culture of *Acidithiobacillus* and *Leptospirillum spp.* and a culture containing only *Sulfolobus spp.* was used to investigate the effects of attachment on the dissolution of pyrite, chalcopyrite and sphalerite. A correlation between attachment and dissolution rate was observed, supporting higher dissolution rates on higher initial levels of bacterial attachment.

Kinzler *et al.* (2003) investigated the structure and function of extra-cellular polymeric substances, excreted by *A. ferrooxidans*, in bioleaching of pyrite. Attachment of *A. ferrooxidans* to pyrite surfaces was analysed using atomic force microscopy, and the rate of pyrite dissolution measured. *A. ferrooxidans* strains which excreted the most EPS, exhibited the highest rate of pyrite dissolution. However, the amount of EPS produced did not correlate with oxidation activities for all strains. Strains excreting EPS with high ferric ion content exhibited higher pyrite dissolution rates. It was proposed that the complexation of ferric ions through uronic acid or other residues at the mineral surface enhances the rate of mineral oxidation by ferric ions, consequently enhancing overall leach rates. Harneit *et al.* (2005) conducted a similar study; however, they expanded the range of microorganisms and mineral substrates used. The investigation involved the adhesion of *A. ferrooxidans, A. thiooxidans* and *L. ferrooxidans* to pyrite, chalcopyrite, galena and sphalerite, with quartz used as a control. Attachment and biofilm formation was visualised using atomic force microscopy and fluorescence microscopy and the oxidation activity measured. Again, cells which contained greater ferric ion content in the EPS exhibited a higher oxidation activity than those with lesser ferric ion contents.

2.6 ADVANTAGES OF ATTACHMENT TO SOLIDS SURFACES

The adherence of bacteria to surfaces is important with respect to the microbial ecology, effective microbial colonisation, and biofilm formation. Biofilms are advantageous in harsh environmental conditions as they provide a favorable microenvironment (Watnick and Kolter 2000) and of protection against various adverse conditions. In the case of medically relevant bacteria such as *Pseudomonas*, bacteria are particularly resistant to conventional antimicrobials as the EPS matrix physically restricts diffusion of these agents effectively shielding bacteria within (Bhinu 2005).

EPS also provides protection against hydrodynamic shear. Purevdorj-Gage and Stoodley (2004), demonstrated that biofilms grown in high shear conditions had a stronger EPS matrix and subsequently more strongly adhered cells than those grown under low shear. In a bioleach heap environment where channelling and hence hydrodynamic shear may vary, the formation of biofilms and EPS would be beneficial. EPS has also been shown to provide significant protection against reactive oxygen species and heavy metals (Starkey *et al.* 2004). These may be beneficial in a bioleach environment

In addition to the advantage of creating a favorable niche and a means of protection within the harsh environment of the bioleach heap, attachment and biofilm formation by bioleach microorganisms is expected to convey an added advantage. In bioleach environments, microbial attachment, in an EPS biofilm is expected to play an important role in the dissolution process. Bacteria attach preferentially to nutritive surfaces when the surrounding nutrient environment is poor to ensure that they maximize access to nutrients in both nutrient poor and rich environments (Watnick and Kolter 2000). In a bioleach environment where ferrous iron availability may be low, it is advantageous to be situated in close proximity to the source of the ferrous ions, namely the mineral surface (Watling 2006). The complexation of ferric ions through uronic acid and other residues within the EPS (Gehrke *et al.* 1998), concentrates reactants required for mineral dissolution. Further the energy generation through oxidation of ferrous iron is in close proximity for iron oxidizing microbes. This may have arisen as a competitive advantage.

Another advantage is that the formation of biofilms allows an increased probability for the acquisition of new environmental survival factors via horizontal gene transfer. This beneficial aspect of biofilm formation has not been explored in bioleach microorganisms (Watnick and Kolter 2000).

2.7 DETACHMENT OF BIOLEACH MICROORGANISMS

While detachment is known to occur and plays an important role in the life-cycle of biofilms, little is known regarding the mechanisms underlying the detachment process. Detachment of microbes from biofilms could be attributed to physical forces such as hydrodynamic shear either via erosion of single cells or sloughing off of large aggregates of biomass (Bryers *et al.* 2004, Purevdorj-Gage and Stoodley 2004), as well as various other stresses. Detachment may also result from biological action such as enzymatic dissolution of the polysaccharide matrix (Allison *et al.* 2004).

The phenomenon of 'hollowing out' of biofilms is also cited in terms of detachment. This refers to microorganisms actively vacating the interiors of the biofilm. However, it may also result from lysis of cells within the interior of the colony (Purevdorj-Gage and Stoodley 2004).

Rojas-Chapana *et al.* (1998) demonstrated that Tween 80 disrupted adhesion of *A. ferrooxidans* to sulfur prills. Arrendondo *et al.* (1994) observed that EDTA affects attachment by destabilising the outer membrane of cell.

2.8 GAPS IN PREVAILING LITERATURE WITH RESPECT TO ATTACHMENT OF MICROORGANISMS IN BIOLEACH ENVIRONMENTS

Early literature regarding attachment of bioleach microbes to mineral surfaces is restricted to the proposal and understanding of a mechanism of attachment, discussed in Section 2.4 (Zobell 1943; van Loosdrecht *et al.* 1990). Studies into cell surface as well as mineral surface chemistry were conducted to explain the observed attachment. These studies suggested that initial attachment is due to hydrophobic and electrostatic interactions between the microbial cell and mineral surfaces (van Loosdrecht *et al.* 1990, Devasia *et al.* 1993, Arrendondo *et al.* 1994 and Blake *et al.* 1994). The role of a cell surface protein was also proposed (Blake *et al.* 2000). Knowledge regarding the role of chemotaxis in attachment of bioleaching microorganisms to mineral surfaces was suggested but has since not been proved explicitly (Rojas-Chapana *et al.* 1998, Jerez *et al.* 2001). The involvement of quorum sensing in the establishment of biofilms in bioleach environments was suggested (Farah *et al.* 2005). There is a requirement for knowledge regarding colonisation of bioleach microorganisms in heap environments.

The focal point of the prevailing body of literature with respect to microbial attachment in bioleach environments has shifted recently toward determining where microbes attach, as opposed to how they attach. Several authors investigated the prevalence of selective attachment of microbes to sulfide minerals over other minerals and used the proposed mechanism of attachment to explain the results observed (Ohmura *et al.* 1993, Nagoaka *et al.* 1999, Gonzalez *et al.* 1999, Sampson *et al.* 2000, Rodriguez *et al.* 2003 and Harneit *et al.* 2005), discussed in Section 2.4.8. However, the body of knowledge regarding selective attachment is limited and is restricted to pure culture experiments involving *A.ferrooxidans*. Sampson *et al.* (2000), Rodriguez *et al.* (2003) and Harneit *et al.* (2005) report observations using mixed culture experiments. Sampson *et al.* (2000) and Rodriguez *et al.* (2003) are the only studies that could be found that investigate selective attachment of thermophiles; however, their observations were contradictory. In addition, the experimental setups of these studies do not provide an adequate *in situ* assessment of the microbial attachment in heap environments.

Studies which investigate the influences of microbial attachment in bioleach environments include the effects of solution chemistry on attachment behaviour, discussed in Section 2.4.7. These investigations involved assessing the effects of growth media on the composition of EPS and thus attachment (Gerhke *et al.* 1998, Harneit *et al.* 2005, Kinzler *et al.* 2003), as well as the effect of pH (Solari *et al.* 1992), redox potential and ferrous and ferric iron concentration (Hansford *et al.* 2001, Ohmura *et al.* 1993, Gonzalez *et al.* 1999). The effect of mineral surface properties on attachment of bioleach microorganisms has been observed (Gerhke *et al.* 1998, Rojas-Chapana *et al.* 1998, Sanhueza *et al.* 1999, Gonzalez *et al.* 1999 and Harneit *et al.* 2005). No integrated studies assessing the combined effects of mineralogy as well as solution chemistry on attachment behaviour of bioleach microorganisms have been conducted. In addition, a comprehensive body of literature of factors which influence attachment behaviour of bioleach microorganisms is lacking. As a result, there is insufficient knowledge available on the detachment of bioleach microorganisms and the factors that influence this occurrence.

The involvement of microorganisms in bioleaching is currently proposed by the indirect contact mechanism (Crundwell 2003), discussed in Section 2.1.3. According to the indirect contact mechanism, attachment of microbes to mineral surfaces and excretion of an EPS layer is important in the bioleaching process. The EPS layer provides a reaction space for mineral dissolution to occur (Gerhke *et al.* 1998) and brings the reactants for mineral dissolution in close proximity to the mineral surface and the microbial cells (Rawlings 2004). In appreciation of this proposed role of attachment and EPS excretion in bioleaching, one could surmise that the state of cells being attached or planktonic would have an effect on the observed bioleach rate. Several authors have correlated attachment of *A.ferrooxidans* to various sulfide mineral surfaces with an increase in bioleach rate (Gerhke *et al.* 1998; Fowler *et al.* 1999; Kinzler *et al.* 2003). However, the effect of the attachment of mixed cultures on bioleach rates is limiting, with only two groups conducting such studies, namely Rodriguez *et al.* (2003) and Harneit *et al.* (2005).

Mineralogy of ores and concentrates affects bioleaching efficiency (Watling 2006) as it affects the leach chemistry, and may result in the formation of products which hinder the bioleaching process, discussed in Section 2.3. The prevailing body of literature does not provide adequate assessment on the effect of mineralogy on heap bioleaching. A study which integrates mineralogical and microbiological effects on bioleaching is required.

The microorganisms involved are acidophilic chemolithotrophic bacteria and archaea (see Section 2.2). However, only a limited number of species have been isolated from bioleach environments. This may be chiefly due to the difficulty associated with current enrichment and culturing techniques (Watling 2006). As a result there is a requirement for studies on microbial diversity and the discovery of novel bioleach microorganisms. Knowledge on the relationships between microbial relationships within bioleach heaps and symbiotic relationships between microbial strains in mixed cultures are currently not well-understood (Watling 2006). A greater understanding of microbial succession[4] within heap environments is therefore required. There is therefore a need for studies, which involve quantification of microbial population compositions and elucidation of strain-specific activities in response to changes within a bioleach environment.

The limitations of the current body of literature and requirement for its extension can be summarised as follows:

- There is a requirement for knowledge regarding colonisation of bioleach microorganisms in heap environments
- The body of knowledge regarding selective attachment is limited and is restricted to pure culture experiments involving *A.ferrooxidans*
- No integrated studies assessing the combined effects of mineralogy as well as solution chemistry on attachment behaviour of bioleach microorganisms have been conducted
- The current body of literature on factors that influence attachment behaviour of bioleach microorganisms is lacking
- There is insufficient knowledge available on the detachment of bioleach microorganisms and the factors that influence this occurrence
- The prevailing body of literature does not provide adequate assessment on the effect of mineralogy on heap bioleaching
- A study which integrates mineralogical and microbiological effects on bioleaching is required
- More information on the effect of the attachment of mixed cultures on bioleach rates is required
- Microbial relationships within bioleach heaps, including symbiotic relationships between microbial strains in mixed cultures, are currently not well understood. These impact our understanding of microbial succession within heap environments. Studies are needed to describe quantitatively, microbial community structure and elucidate strain-specific activities in response to changes within a bioleach environment.

[4] Microbial succession refers to the "selection and development of sequential microbial populations in a natural or disturbed environment" (Martino D.,http://www.woodrow.org/teachers/esi/1999/princeton/projects/microbe/index.html , retrieved 20-10-2009)

2.9 OBJECTIVES, KEY QUESTIONS AND HYPOTHESES OF THIS STUDY

2.9.1 Objectives and scope of research

Stemming from a requirement to address the gaps in the current body of literature regarding microbial attachment and the role of microorganisms in heap bioleach environments, and to mitigate challenges faced by industry through the investigation of microbial attachment and colonisation under heap-like conditions, this study was born. The aim of this study was to investigate attachment of three bioleach microorganisms (*A. ferrooxidans, L. ferriphilum* and *S. metallicus)* to sulfide minerals in a bioleach environment using methodologies simulating heap-like conditions. It also served to develop a novel integrated *in situ* technique for the investigation and visualisation of the spatial microbial attachment of microbes to mineral surfaces both in terms of the arrangement of mixed microbial communities and the location of microbes on the mixed mineral surface. This encompassed the use of pure and mixed cultures, as well as pure and low-grade chalcopyrite mineral containing ores, under heap-like fluid flow dynamics.

The purpose of the research was to investigate the attachment of microorganisms commonly isolated from bioleach environments to sulfide mineral surfaces, with the following objectives:

- To investigate trends in microbial attachment of *A. ferrooxidans, L. ferriphilum* and *S. metallicus* to a pyrite mineral concentrate, chalcopyrite mineral concentrate, low-grade chalcopyrite containing mineral ore, using quartz as an inert control mineral in batch agitated and packed column reactors, to validate the approaches used and assess the applicability of these approaches to a heap environment
- To assess the effects of growth history on surface charge and subsequent trends of microbial attachment to sulfide minerals using the packed column reactor
- To elucidate the prevalence with which bioleach microorganisms attach 'preferentially' to sulfide minerals under these experimental conditions
- To investigate the influence of solution chemistry on the level of attachment observed by considering the pH, high ionic strength and redox potential.
- To demonstrate and validate the development of a novel, integrated *in situ* technique for the study of microbial attachment onto mixed mineral surfaces including spatial considerations using the biofilm reactor
- To draw conclusions on observed trends in microbial attachment using the various methodologies

2.9.2 Key questions

- How do microorganisms attach to solid surfaces? (Addressed in literature review)
- Do microorganisms attach preferentially to sulfide minerals? Do they attach elsewhere as well? And if so where?
- If preferential attachment occurs, is it specific on a microbial species basis?
- Is there preferential attachment to specific locations, such as visible defects on the mineral surface?
- Is there any observed spatial arrangement in the attachment?
- What factors influence the attachment of microbes to the mineral surface?
- What influences the detachment of microbes from the mineral surface?
- Do variations in growth history or culture conditioning using various substrates alter cell surface properties?
- Can these variations in culture conditioning and subsequent changes in cell surface properties be correlated to a change in subsequent microbial attachment behavioural trends?

2.9.2 Development of Hypotheses

Primary adhesion of microorganisms to mineral surfaces may be due to non-specific binding explained by hydrophobic and electrostatic interaction between the cell surface and minerals (van Loosdrecht *et al.* 1990, Devasia *et al.* 1993, Arrendondo *et al.* 1994 and Harneit *et al.* 2005). However, there have been reports of selective adhesion by *A. ferrooxidans* to sulfide mineral surfaces. Harneit *et al.* (2005), Rodriguez *et al.* (2003), Sampson *et al*. (2000), Gonzalez *et al.* (1999), Nagaoka *et al.* (1999) and Ohmura *et al.* (1993), all demonstrated selective attachment of *A. ferrooxidans* to sulfide mineral surfaces over other minerals. The attachment of *A. ferrooxidans* around surface imperfections was reported by Gehrke *et al.* (1998), Rojas-Chapana *et al.* (1998) and Harneit *et al* .(2005). However, rigorous data for other species and mixed cultures and gangue mineralogy are limited. Furthermore, agitated batch methodologies employed (shake flasks) are ill representative of bio-heap fluid dynamics. This leads to the development of the first hypothesis which is investigated by extending methodologies to encompass a continuous flow through packed column reactors, an *in situ* biofilm reactor system, gangue containing sulfide mineral ores, sulfide mineral concentrates and mixed microbial consortia.

Hypothesis 1:

Bioleach microorganisms attach selectively to sulfide mineral surfaces over other mineral surfaces, and attach preferentially to surfaces with visible defects under conditions representative of a bio-heap.

Initial attachment of microorganisms to solid surfaces are believed to be governed by transient, reversible electrostatic and hydrophobic interactions between the mineral and microbial surfaces (Zobell 1943, van Loosdrecht *et al*. 1987, van Loosdrecht *et al.* 1990, Rijnaarts *et al.* 1993, Porro *et al.* 1997, Blake *et al.* 1994, Nagaoka *et al.* 1994, Sampson *et al.* 2000, Sharma *et al.* 2003), dependent on mineral and microbial surface properties (surface charge and hydrophobicity). The effect of culture conditioning on the alteration of cell surface properties has been reported by Porro *et al.* (1993), Rijnaarts *et al.* (1993), Blake *et al.* (1994), Gerhke *et al.* (1998), Kinzler *et al.* (2003), Sharma *et al.* (2003) and Harneit *et al.* (2005). This led to the following hypothesis.

Hypothesis 2:

Growth conditioning of microorganisms affects cell surface properties (such as cell surface charge) and affects subsequent microbial attachment behavioural trends under conditions representative of a bio-heap. Enhanced levels of microbial attachment are predicted for mineral adapted microorganisms relative to ferrous grown cultures under these conditions.

Three approaches were employed in this study for investigation of these hypotheses:

- Pure culture, agitated batch systems using shake flasks, as previously used in literature studies
- Pure culture, continuous fluid flow systems using packed column reactors
- Pure and mixed culture, continuous flow systems using a biofilm reactor

Chapter 3: Methodology

In this chapter, a description of protocols and techniques employed to investigate attachment is presented with special regard to experimental set-up and reactor configuration. Mineralogical information of the substratum used is provided in Section 3.1. The microbial species used and their respective growth conditions are presented in Section 3.2. Descriptions of the reactor configurations are provided in Section 3.3. Analytical and molecular techniques employed are given in Sections 3.4 through 3.9. Finally, descriptions of the experimental design used to study attachment are given in Section 3.10.

3.1 SUBSTRATA USED TO INVESTIGATE ATTACHMENT

3.1.1 Sulfide mineral substratum

A pyrite mineral concentrate (Agnes mine, courtesy of BHP Billiton) and a chalcopyrite (79.52 wt %) mineral concentrate (Andina mine, courtesy of BHP Billiton) were used. A summary of the major elemental composition of the concentrates, determined by X-ray fluorescence (XRF) analysis (analysis performed as uncalibrated samples courtesy of Mrs. H. Divey, Department of Chemical Engineering, University of Cape Town) is presented in Table 3.1.The samples were milled and wet sieved to size fractions between 38 μm and 75 μm. Particle size distribution was performed by laser diffraction using a Malvern Mastersizer (Malvern Instruments) particle size analyser. The results showed a final size fraction less than 30 μm was achieved for both the pyrite mineral chalcopyrite mineral concentrates (Appendix E).

Table 3.1: Major elemental composition of the Agnes pyrite and Andina chalcopyrite concentrates.

Mineral concentrate	Copper (%)	Iron (%)	Sulfur (%)
Chalcopyrite	29.8	25.9	31.7
Pyrite	0.197	26.6	14.8

A low grade run of mine ore (CH-32 Type B, Escondida, courtesy of BHP Billiton) was milled and wet sieved to a final size fraction between 38 μm and 75 μm. The predominating sulfide minerals present were pyrite and chalcopyrite (4.0 and 0.5 wt %, respectively), with the predominating gangue minerals present being quartz and muscovite (44.8 and 28.6 wt %, respectively). The full mineralogical composition of the CH-32 Type B ore is presented in Appendix E.

3.1.2 Quartzite

Quartzite (Consol Glass, Cape Town), was milled and wet sieved to a final size fraction between 38 and 75 µm. This mineral served as an inert control mineral for the purpose of the particle packed column reactor attachment studies.

3.1.3 Mineral coated glass beads for column attachment study

The particle packed column attachment studies required quantifiable and unform mineral surfaces. In order to accomplish this, glass beads (Lasec, South Africa) 6 mm in diameter, were coated with the four minerals under investigation using bostik clear adhesive and subsequently air dried for a minimum of 24 hours. An even, uniform coverage of the entire bead surface was maintained. This particular adhesive is stated to be stable under aquatic conditions and the adhesive is assumed to have a negligible impact of attachment of microorganisms as even coverage of the entire bead surface with the mineral was ensured. Surfaces of all four minerals were created with freshly coated beads were used in each experiment.

3.1.4 Preparation and mineralogical mapping of mineral thin sections for biofilm attachment study

A predominantly chalcopyrite rock sample and a low grade chalcopyrite mineral containing ore sample (Escondida, Chile) were used. For experimental studies, the samples were cut into 60 µm and 100 µm thin sections, and mounted onto glass slides using an epoxy resin (Department of Geology, University of Cape Town). The 60 µm thin sections were polished for the purposes of generating a mineralogical map, with the 100 µm thin sections left unpolished to investigate the prevalence of microbial attachment to certain surface properties such as roughness and the presence of surface defects.

Important properties which guide the identification of specific minerals include the physical properties of the mineral as well as optical properties. The physical properties which are commonly used for mineral identification include properties such as hardness, tenacity, streak, lustre, density and specific gravity, cleavage, parting, fracture and associated minerals (Rogers 1937). Hardness refers to the resistance offered by the smooth surface of a mineral to scratching. The property is dependant on the strength and atomic bonding in the mineral. Tenacity refers to 'the resistance offered by a mineral to breaking crushing, bending or tearing' also referred to as its 'cohesiveness'. Streak refers to the colour of finely powdered mineral. Cleavage refers to the tendency of certain minerals to break smoothly, parallel to particular planes of atoms in the crystal structure. The presence of cleavage therefore indicates weak bond strength in the crystal along those planes. Parting is a description of the tendency of a mineral to break along discrete surfaces of structural weakness. Of the physical properties presented for mineral identification, only mineral associations were considered. Other factors were less important since the basis of the mineral identification technique employed relied on classification of minerals through characteristic optical properties via ore microscopy.

Optical properties refer to properties such as refractive index, luminescence and colour, as well as pleochroism, isotropism or anisotropism, birefringence, internal reflectance and twinning, all of which may be determined using ore microscopy (Rogers 1937). Colour pleochroism refers to a change in the colour exhibited by anisotropic minerals due to differing degrees of absorption of light in different crystallographic orientations in plane polarized light. Isotropic crystals allow light to move in all directions with equal velocity and have a single refractive index. In cross polarised light these appear dark in all directions. In the case of anisotropic crystals, the velocity varies with crystallographic direction and there is therefore more than one refractive index. In cross polarised light these show interference colours (associated with birefringence) with characteristic extinction. Birefringence refers to the difference in refractive index. Minerals will exhibit a range of colours due to strong birefringence under transmitted, cross polarized light. The Michel-Levy chart is used as a standardized means to reveal the characteristic colours exhibited by a mineral according to its birefringence index (Appendix E). Twinning refers to the occurrence of two separate crystals sharing the same crystal lattice points in an asymmetrical manner, which results in an intergrowth of the crystals in a variety of specific configurations.

Ore microscopy exploits the optical properties of various minerals under reflected and transmitted light in polarised and cross polarised planes in order to identify minerals present. Ore microscopy, together with mineralogical information provided by BHP Biliton Escondida mine, was used to characterise ore samples and identify minerals present. Using this technique, together with photographic analysis, a data base was compiled for the generation of a detailed mineralogical map of the surface of each thin section studied. Minerals present were identified, labelled and mapped in order to identify the type, spatial arrangement, and relative abundance. Table E.3 in Appendix E shows the predominant sulfide and gangue minerals contained in the ore sample with their corresponding optical properties used for mineral identification via this technique.

The thin section surface micrographs were captured using a Zeiss polarising geology microscope attached to a Canon camera. Images were managed using Zoombrowser-X Canon imaging software. Photographs were taken in transmitted and reflected light, in polarised and cross-polarised planes, using various magnifications (5 x, 10 x and 50 x objectives).

3.2 MICROBIAL CULTURES

3.2.1 Ore free culture conditions

Three microbial species were investigated; namely *A. ferrooxidans, L. ferriphilum* and *S. metallicus.* Microorganisms were cultivated in defined media (Appendix A). *A. ferrooxidans* and *S. metallicus* were cultivated in Erlenmeyer flasks on orbital shakers operating at 160 rpm. *A. ferrooxidans* was cultured with ferrous sulfate as an energy source, at 30℃ and pH 2.0. *S. metallicus* was cultured with elemental sulfur serving as an energy source at 65℃ and pH 2.0. *L. ferriphilum* was cultured in a continuous stirred tank reactor (CSTR) with ferrous sulphate as energy source at 37℃ and pH 1.8. Sub-c ulturing of microorganisms grown under shake flask conditions took place twice weekly with 30 % of the liquid volume in the flask, removed and replace with fresh media. Sub-culturing was done under sterile conditions, by flaming the mouth of all feed and culture containing vessels at each sub-culturing event, in a fume hood. Morphology of microorganisms was routinely monitored using an *Olympus* microscope. Due to the frequency of sub-culturing, it was assumed that microorganisms remained in an actively growing state. Microbial growth kinetics was not monitored.

3.2.2 Mineral adapted culture conditions

Ore free cultures of *A. ferrooxidans* and *L. ferriphilum* were weaned onto a suspension of 9 K media at pH 2.5 containing 0.67 % (wt vol^{-1}) pyrite mineral concentrate. The weaning procedure involved the addition of 2 g of a pyrite concentrate to an actively growing ore free microbial culture having a final liquid volume of 300 ml. This culture was then sub-cultured twice a week for one month with 30 % of the actively growing culture removed and the volume of the solution made up by the addition of 9 K media at pH 2.5. A mass of 0.5 g of a pyrite concentrate was added at each sub-culturing event to account for any loss of mineral during the sub-culturing procedure. These cultures were deemed sulfide mineral (pyrite) “mineral adapted” cultures. Mineral adapted cultures were maintained through sub-culturing twice weekly with the volume made up with 9 K media and the addition of 0.5 g of pyrite to account for the loss of any mineral during the sub-culturing event.

3.3 REACTOR CONFIGURATION

3.3.1 Particle packed column reactor configuration

Three identical glass columns were constructed (Glasschem, Stellenbosch), with each column having a 2.5 cm diameter and a 19 cm working length. The ends of each reactor were sealed with screw-cap lids containing a rubber stopper, to prevent any leakages, to which a glass nipple was connected. A peristaltic pump was connected to the glass nipple at the bottom of the reactor, and ensured delivery of feed medium at a constant rate of 1ml min^{-1}. Each column was loaded with 300 mineral coated beads (6 mm in diameter) and saturated with 9 K medium from the bottom. The bottom inlet of each column was covered with glass wool, and 100 smaller beads having an average diameter of 4 mm. This was done to ensure uniform distribution of feed into the column. The residence time of a packed column was calculated in duplicate using step introduction of a pH tracer (using 1 x phosphate buffer saline (PBS) solution at pH 7.0 and H_2SO_4 at pH 2.0) and was determined to range between 52 to 56 minutes (Appendix C). The void volume of the reactor was approximately 55 ml. The column did not exhibit perfect plug flow. The dimensionless Peclet number (Pe_L) was calculated to be 0.03 which is indicative of dispersion within the column and thus deviations from perfect plug flow (Levenspiel O., see Appendix G). The fluid flow within the column fell within the laminar regime as the Reynolds number was calculated to be approximately 0.267 (Felder R. M., Rousseau R. W., see Appendix G).

3.3.2 Biofilm reactor configuration

The biofilm reactor consisted of sealed circular vessel, having a 10 cm outer diameter, in which the thin section was housed. The reactor was connected to a peristaltic pump which delivered feed as an evenly distributed film across the surface of the ore section through the use of specially designed nozzles at a rate of 60 µl min^{-1}. The ore sections were mounted at a slight decline to ensure one way fluid flow through the reactor. The reactor was operated as a closed recycle for the duration of the inoculation period, after which it is run as a continuous flow through system, with fresh 9 K medium (pH 2.5) introduced continuously and eluted samples collected.

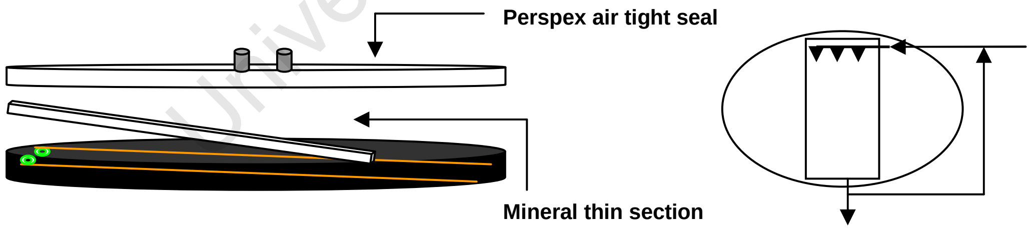

Figure 3.1: Schematic representation of the biofilm reactor configuration (left) and fluid flow operation (right).

The biofilm reactor was housed in a fume hood in order to minimize the risk of contamination when conducting pure culture experiments. A schematic representation of the biofilm reactor configuration (left) and fluid flow operation (right) is presented in Figure 3.1. The residence time was calculated to be 5.6 hours (see Appendix F for calculations).

3.4 ANALYTICAL TECHNIQUES

3.4.1 Determination of pH

All pH measurements were performed using a Metrohm 704 pH metre and probe, which was calibrated at pH 7.0, pH 4.0 and pH 1.0 each time before use.

3.4.2 Redox potential analysis

All redox potential readings were determined using a Metrohm 704 pH (Ag/AgCl reference electrode). The precision of the measurements was tested using a Crison standard redox solution having a potential of 468 mV at 25˚C.

3.4.3 Conductivity readings

Conductivity readings were measured using a Metrohm (pH, redox and conductivity) metre and probe. The precision of the measurements was tested using a KCl standard (Metrohm) conductivity solution at room temperature.

3.5 ZETA-POTENTIAL MEASUREMENTS

The surface charge of ore free and mineral adapted microbial species was measured using a Malvern zeta sizer. The zeta potential of the cells was measured in a cell culture with a concentration of a 2 x 10^8 cells ml^{-1} suspended in 9 K medium at pH 2.5. Readings were carried out at pH 2.5 since attachment studies had been conducted at this pH. A minimum of four measurements was taken per sample.

3.6 ENUMERATION OF CELL CONCENTRATION

The concentration of the inoculum (cells ml^{-1}) was determined microscopically using a Thoma counting chamber and an *Olympus* epifluorescent microscope using 1000x magnification (under oil emersion). The formula used for calculating cell concentration per ml using microscopy and a Thoma counting chamber was as follows:

Volume of one small square

$$= depth * area = \frac{0.02 * (0.05 * 0.05)}{1000} = 5x10^{-8} cm^3 \quad \text{(Eqn 6)}$$

Concentration total (cells ml^{-1})

$$= dilution\ factor * \frac{(cell\,count * \frac{N}{n})}{volume\,one\,suqare * total\,number\,of\ small\,sqaures} \quad \text{(Eqn 7)}$$

Where N = total number of big squares (16),
n = number of squares counted (4).

3.7 INOCULUM PREPARATION USED FOR ATTACHMENT STUDIES

Cultures were centrifuged at 2000 rpm (JA 20 rotor, Beckmann centrifuge), under ambient temperature conditions to remove precipitates if necessary. Cells were then harvested by centrifugation at 10 000 rpm (JA 20 rotor, Beckmann centrifuge) at 4 ℃. The pellet was re-suspended in 9 K medium (see Appendix A) at pH 2.5. The cell concentration was then determined as per section 3.3. The inoculum was then diluted, if necessary, with 9 K medium in order to obtain the desired cell concentration.

3.8 FLUORESCENT *In Situ* HYBRIDISATION (FISH)

In this study the FISH technique was employed in the biofilm reactor approach to visualise and differentiate between the attachments of various microorganisms to thinly sectioned mineral surfaces *in situ*. Probes were fluorescently labelled with 6-FAM and purified by high-pressure liquid chromatography (Synthetic DNA laboratory, University of Cape Town*)*. The oligonucleotide probe LF581 was used in this study, the sequence of which is presented in Table 3.2, and is specific to the genus *Leptospirillum*. The specificity of the probe has been assessed elsewhere (Coram-Uliana *et al.* 2006).

Table 3.2: Sequence of the oligonucleotide LF581 probe used

Probe	Target group	Sequence	Reference
LF581	Genus: *Leptospirillum*.	5'-CGGCCTTTCAC-CAAAGAC-3' (*E. coli positions* 581-598)	Schrenk *et al.* 1998

The FISH protocol employed was adapted from Coram-Uliana *et al.* 2006. Once colonised, the mineral ore thin section was removed from the biofilm reactor, washed gently with distilled water and dried at 46 ˚C. The cell walls of the sample were permeabilised and the cell DNA was made single stranded. This was accomplished by incubation of the sample on ice for 20 minutes with 10 µl lysozyme (10 mg ml^{-1}). Subsequently the section was washed with ice cold distilled water. A dehydration step followed using an increasing ethanol series (50 %, 80 %, 100 % ethanol with a 3 minute exposure in each). Following this the probe was allowed to hybridise with the sample DNA. Hybridisation and wash buffers contained 30 % deionised formamide. The sample was hybridised for 2 hours at 46 ˚C in the dark using 10 µl hybridisation buffer containing 50 ng fluorochrome-conjugated probe. Once hybridisation was complete, the sample was washed for 15 minutes in preheated wash buffer (48 ˚C) and then rinsed with ice cold distilled water to remove excess probe. Finally, the sample was counter stained for 10 minutes using 10 µl of a 1µg ml^{-1} 4', 6-diamidodino-2-phenlyindole solution (DAPI, Sigma Chemical Co), rinsed with distilled water and dried using compressed air. The surface of the sample was then embedded using mounting medium (Citifluor™). The fluorescence was visualised using an *Olympus* epifluorescent microscope using ultraviolet (UV) and interference blue (IB) filters, allowing visualisation of DAPI (excitation λ 372 nm and emission λ 456 nm) and 6-FAM labelled probe (excitation λ 494 nm and emission λ 519 nm) fluorescence respectively. Images were captured and managed using the Analysis® Soft Imaging System.

3.9 DATA ANALYSIS

The following section presents a description of the method used to quantify attachment efficiency (Section 3.9.1) and the attachment per surface area available (Section 3.9.2).

3.9.1 Quantification of "attachment efficiency"

Unless otherwise stated, 'attachment' efficiency is presented as the percentage of cells retained in the reactor vessel at the end of an experiment. The cumulative proportion of cells retained in the reactor vessel was quantified by subtracting the cell number of eluted samples from the inoculum cell number and can be given by the following equation:

$$\text{Attachment efficiency} = \left(\frac{Co - C}{Co}\right) * 100 \qquad \text{(Eqn 8)}$$

Where:

Attachment efficiency = cells retained in the reactor vessel (%)
Co = inoculum total cell number
C = total cell number in eluted samples (cumulative)

The calculation did not distinguish between the level of attachment to the vessel wall and glass beads. It was assumed that attachment to vessel walls is negligible based on findings of Ohmura *et al.* (1993).

3.9.2 Quantification of maximum attachment per unit surface area

Quantification of the maximum attachment per available surface area provided information on the degree of surface saturation by the inoculum. Saturation of the surface available for attachment by the inoculum may influence the final attachment efficiencies observed. Thus, these calculations allowed the impact of inoculum concentration on the attachment to be analysed and allowed more adequate comparison of extent of attachment across experiments.

The following assumptions were made to calculate the saturation concentration:

- Repulsive forces between cells and minerals were ignored

- Attachment was monolayered with cells orientated such that the major axis was parallel to the surface of the mineral coated bead
- Dimensions of a mesophilic microorganism: 1.5 x 0.5 µm (Ohmura *et al.* 1993)

The total surface area made available by the 300 spherical, mineral coated, glass beads, 6mm in diameter, housed in the reactor was calculated to be 3.4 x 10^{-2} m^2. The maximum number of *A. ferrooxidans* cells that could be accommodated by the available surface area in the reactor was calculated to be 4.5 x 10^{10} cells. These calculations are given in Appendix B. This was done by dividing the total surface area made available for attachment by the mineral coated glass beads, by the surface area per microbial cell. This number will be referred to as the saturation cell number.

Attachment efficiency per unit surface area was performed by dividing the cumulative proportion of cells retained in the reactor by the total surface area available for attachment as shown in Equation 9 and detailed in Appendix B.

$$= \frac{Cumulative\ number\ of\ cells\ retained\ in\ the\ reactor\,[cells]}{Total\ surface\ area\ made\ available\ for\ attachment\ by\ coated\ glass\ beads\,[m^2]}$$

(Eqn 9)

3.10 EXPERIMENTAL DESIGN AND RATIONALE

3.10.1 Agitated batch systems using shake flasks

To date, the most widely used means of investigating attachment related to heap bioleaching has been through the use of batch agitated systems (Ohmura *et al.* 1993, Gehrke *et al.* 1998 and 2001, Nagaoka *et al.* 1999, Gonzalez *et al.* 1999, Sampson *et al.* 2000, Rodriguez *et al.* 2003, Kinzler *et al.* 2003 and Harneit *et al.* 2005) discussed in Chapter 2. The work presented in this chapter was done with the purpose of investigating attachment behaviour of bioleach microorganisms using the approach employed in literature; and to assess whether or not this approach provided an adequate representation of attachment in a heap bioleach environment.

The attachment behaviour of three ore-free cultured microbial suspensions (*A. ferrooxidans, L. ferriphilum and S. metallicus*) to a pyrite containing mineral concentrate, a chalcopyrite containing mineral concentrate, a low grade chalcopyrite containing mineral ore and quartz, was investigated in batch agitated systems using shake flasks. The prevalence of selective attachment was also assessed. These attachment experiments were conducted in 250 ml Erlenmeyer flasks under aseptic conditions. Minerals were washed three times using acetone, followed by five washes with acidified water (H_2SO_4 pH 1.8), using a vortex machine.

Subsequently the mineral was dried in an oven at 80 ℃. Flasks containing 90 ml were inoculated using 10 ml of a pure culture inoculum (prepared as per section 3.2) yielding a final working volume of 100 ml of a 9 K medium (pH 2.5) and placed on an orbital shaker at 180 rpm in a temperature controlled room at 37° C. A mi neral loading of 2% (wt vol^{-1}) was added to the shake flask. Samples having a 1 ml volume were collected at 1, 5, 10, 15, 20, 30, 60 and 120 minute intervals and the cell concentrations enumerated and attachment efficiency determined using Equation 8.

3.10.2 Particle packed column attachment experimental approach

Hydrodynamic conditions experienced in batch agitated systems differ from those encountered in a heap, an important factor which is overlooked by investigating attachment in batch agitated systems. Shake flask systems present a well-mixed environment which may facilitate more effective particle transport. Stemming from this, the concept of the column attachment approach was born. This approach provided a more representative investigation of attachment in a heap bioleach context as it simulated heap-like dynamics through the use of three identical glass particle packed column reactors (column attachment studies were conducted in triplicate). The glass column reactors were loaded with 300 mineral coated beads, 6 mm in diameter. The use of mineral coated beads allowed a quantifiable surface area on which attachment could occur (the calculations of the total available surface area in a column reactor is provided in Appendix B).

The columns were saturated with 9K media prior to inoculation, with feed introduced from the bottom of the column at a constant rate of 1 ml min^{-1}. The flow rate employed was based on flow rates used in heap bioleach column experiments previously conducted at the Centre of Bioprocess Engineering Research (CeBER) in the Department of Chemical Engineering at the University of Cape Town (Appendix F) which were typically 0.7 ml min^{-1} having a linear velocity of 1.41E-06 m sec^{-1}. The inoculum was introduced as a pulse. Subsequently fresh medium was continuously introduced into the column from the base of the column. Nine samples, from each reactor were collected from the effluent stream in 15 ml aliquots at 15 minute intervals. Cell concentrations of eluted samples were enumerated and the attachment efficiency calculated as previously described. Attachment efficiency represented the percentage of the inoculum which remained in the column at that sample point and was represented graphically against time. Experiments were conducted in triplicate using three identical reactors. Unless otherwise stated, duration of each experiment was 135 minutes, with the residence time of the column calculated to be approximately 56 minutes (Appendix C) with the reactor void volume being approximately 55 ml. The pH, redox potential and conductivity of eluted samples were measured. All eluted samples were enumerated for cell concentration on the same day of the experiment with all analytical procedures were conducted either on the same day or subsequent day of the experiment, in which case samples were stored at 4˚ C.

3.10.3 Biofilm reactor approach

Key points arising from assessment of the available literature on the subject of microbial attachment are summarised in Chapter 2.8. Thus, the aim of this study was to develop a novel, integrated *in situ* approach for the investigation of mineral-microbe interactions encompassing the use of low grade chalcopyrite ores and mixed microbial consortia in an environment simulating heap attachment dynamics in which the spatial attachment of microorganisms to mineral surfaces could be studied. This sought both to establish microbe-mineral interactions and the structure of mixed microbial communities. Thinly sectioned massive or low grade chalcopyrite containing mineral ore mounted onto glass slides were used. These sections were mineralogically mapped using ore microscopy and photographic analysis. The surface of the section was gently wiped with acetone followed by acidified water (H_2SO_4 pH 1.8) and then rinsed with distilled water. Following this, the sections were placed in a biofilm reactor and subsequently inoculated with pure or mixed cultures of *A. ferrooxidans* and *L. ferriphilum*. The inoculum was prepared as described previously. Prior to inoculation, 9 K media was allowed to flow over the surface of the section in order to reduce surface tension, and allow for evenly distributed feed across the surface of the sample once the inoculum was introduced. The design of the reactor allowed feed to be distributed evenly as a thin film over the surface of the mineral thin section. This simulated the trickling fluid flow of the leach liquor percolating through an industrial heap.

Inoculum concentrations used in column studies conducted at the Centre for Bioprocess Engineering Research range from 10^5 to 10^9 cells kg^{-1}.To validate the biofilm reactor, an inoculum having a concentration of 5×10^5 cells ml^{-1} suspended in 20 ml of 9 K medium, of the microorganism of interest, was used in this study. The biofilm reactor was operated as a closed recycle for the inoculation period allowing a contact period of the microorganisms with the mineral surface of approximately 72 to 96 hours for pure culture experiments and 20 hours for mixed culture experiments. Subsequently it was operated as a continuous flow through system using fresh medium for mixed culture experiments. The duration of the experiment ranged from 48 to 96 hours as the technique was still being optimised. A flow rate 300 µl ml^{-1} was used initially in order to demonstrate the technique. Subsequently, a flow rate of 60 µl min^{-1} was used which equates to a linear velocity of 7.8×10^{-5} m s^{-1}. This linear velocity is comparable to that typically found in heap environments, where linear velocities of 1.4×10^{-6} m s^{-1} are reported (Information used for industrial heap linear velocity calculation was supplied courtesy of BHP Billiton. Calculations are found in Appendix E). Assumptions made include perfect plug flow, negligible friction and a constant flow rate of 60 µl min^{-1}.

Once colonised, the thin section was removed from reactor and the attached cells visualised through the use of fluorescent stains (FISH) and various microscopy techniques. The results of the FISH procedure were compared to the mineralogical maps compiled and the spatial locale, distribution and relative abundance of the attached microorganisms assessed.

Chapter 4: Agitated batch attachment study

4.1 INTRODUCTION

From the literature review, it is evident that of the few attachment studies conducted in the bioleach field, most have been carried out in batch agitated systems or shake flasks. In this chapter, the attachment of *A. ferrooxidans* to a pyrite concentrate, chalcopyrite concentrate and low grade chalcopyrite milled ore sample, with quartz used as a control mineral was assessed using batch agitated systems as cited in literature. All minerals were milled and wet sieved to a final size fraction between 38 μm and 75 μm, with pyrite and chalcopyrite mineral concentrates having a final average particle size of between 10 μm and 60 μm, and 10 μm and 40 μm respectively. Experiments were conducted in 250 ml Erlenmeyer flasks, using a 2 % mass volume^{-1} suspension of the mineral of interest in 9 K medium at pH 2.5. The experiment was carried out at 30˚ C, on an orbtial shaker operating at 180 rpm. Samples of 1 ml were taken after contacting for 1, 5, 10, 20, 30, 60 and 120 minutes and the planktonic cell concentration enumerated.The attached population was then determined by subtracting the planktonic cell number from the cell number of the inoculum, according to Equation 8.

Trends of attachment of *A. ferrooxidans* to the sulfide minerals (pyrite and chalcopyrite) are presented in Section 4.2. This is followed by presentation of results to interrogate the prevalence of selectivity in the attachment behaviour of *A. ferrooxidans* to sulfide mineral concentrates and gangue, with quartz used as a control. Finally a discussion of the results is given in Section 4.3, with conclusions drawn in Section 4.4.

4.2 RESULTS

A summary of the maximum attachment efficiencies attained for the attachment of *A. ferrooxidans* to the respective minerals in a shake flask experimental set-up is presented in Table 4.1, with the results of the experiment presented graphically in Figure 4.1. The results observed for the attachment of *A. ferrooxidans* to pyrite and chalcopyrite mineral concentrate are presented first.

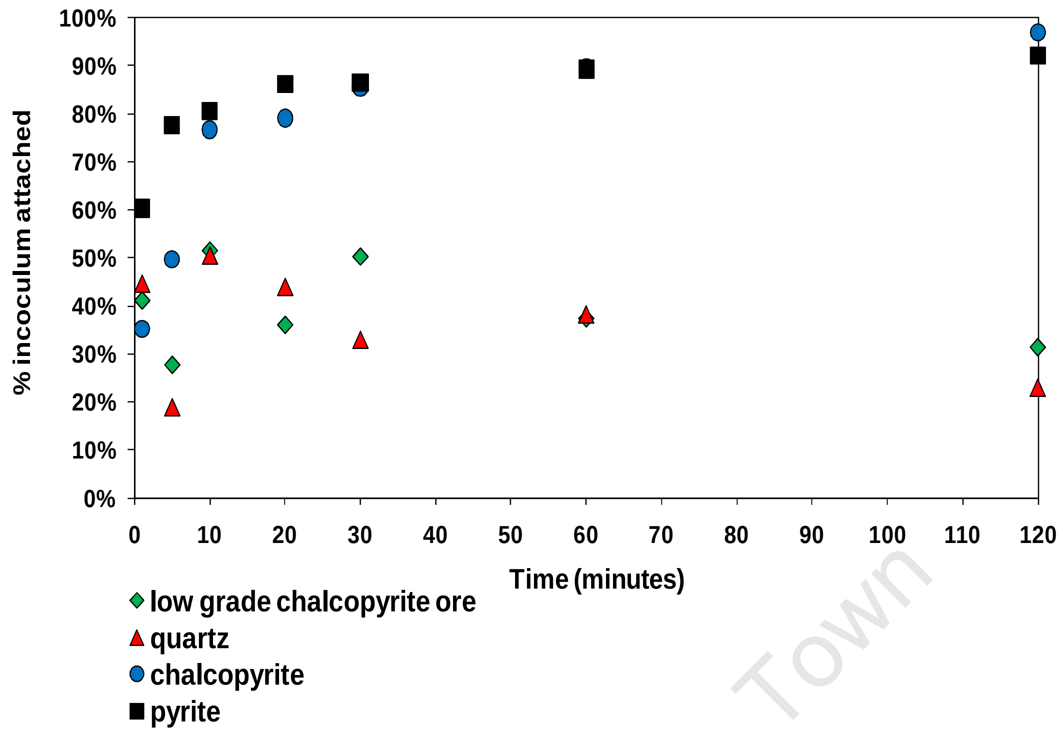

Figure 4.1: Attachment efficiency of *A. ferrooxidans* to pyrite mineral concentrate, chalcopyrite mineral concentrate, a low grade chalcopyrite containing mineral ore and quartz; determined using a shake flask experimental setup. Attachment efficiency represents the percentage of inoculum attached to mineral at the respective sample point.

Upon analysis of the results presented in Figure 4.1, a rapid, high level of attachment of *A. ferroxidans* to both pyrite and chalcopyrite was evident. The equilibrium attachment of 86 % was achieved after 20 and 30 minutes to pyrite and chalcopyrite respectively, thus attachment to pyrite occurred more rapidly. The levels of attachment attained after 30, 60 and 120 minutes increased only marginally, from 86 % to 89 % and 92 % for pyrite; and from 85 % to 89 % and 97 % for chalcopyrite for the given time periods respectively. The levels of attachment to both sulfide minerals are consistently high once equilibrium has been established.

Table 4.1: Maximum attachment efficiency attained for the attachment of *A. ferrooxidans* to the respective minerals in a shake flask experimental set-up. Here the maximum attachment efficiency represents the maximum percentage of the inoculum attached to mineral, which occurred after 2 hours for pyite and chalcopyrite, and after 10 minutes for low grade chalcopyrite containing mineral ore and quartz.

	Mineral			
	Pyrite (FeS_2)	Chalcopyrite ($Cu FeS_2$)	Low grade chalcopyrite	Quartz (SiO_3)
Maximum attachment efficiency (%)	**92**	**97**	**52**	**50**
Attachment (%) after 2 hours	**92**	**97**	**32**	**23**

The maximum attachment efficiency of *A. ferrooxidans* is greatest for the sulfide mineral concentrates, with the attainment of attachment equilibrium evident and greater than 90% attachment achieved after 120 minutes

The high levels of attachment of *A. ferrooxidans* to pyrite and chalcopyrite suggests this affinity of microorganism for attachment to sulfide minerals. In addition, similarities in the initial rate of attachment for *A. ferrooxidans* to these two sulfide minerals, may lead one to infer that the mechanisms involved in attachment of *A. ferrooxidans* to these minerals may be analagous. Adhesion of cells to vessel walls was assumed to be negligible based on the findings of Escobar *et al.* (1996).

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Attachment of *A. ferrooxidans* to low grade chalcopyrite containing mineral ore and to quartz was erratic with no specific attachment trends evident, The levels of attachment to quartz and low grade chalcopyrite containing mineral ore remained within similar range, with between 20 % and 50 % of microorganisms being retained on the solid phase across the 2 hour period. The maximum levels of attachment were 52 % and 50 % to low grade chalcopyrite containing mineral ore and quartz after 10 minutes respectively. Attachment at 2 hours was 32% and 23% to low grade chalcopyrite containing mineral ore and quartz respectively.

4.3 DISCUSSION

Overall attachment to pyrite and chalcopyrite was substantially higher relative to the control mineral quartz and the low grade chalcopyrite ore; with maxima of 97 % and 92 % attachment attained to chalcopyrite and pyrite, and 50 % and 52 % attained to quartz and low grade chalcopyrite containing mineral ore. While the maxima for the sulfide minerals represented equilibrium attachments, for the gangue minerals these decreased to 32% and 23% following 2 hour contact. This provided evidence of preferential attachment of *A. ferrooxidans* to sulfide minerals relative to quartz and low grade chalcopyrite. In addition to the higher levels of attachment observed to sulfide minerals, the initial rate of attachment to these minerals was observed to be rapid with levels of attachment approaching greater than 90 % of the equilibrium value within 30 minutes. The observation of rapid, high levels of attachment of *A. ferrooxidans* to sulfide minerals with the attainment of attachment equilibrium is consistent with literature reports by Atkins *et al.* (1986), Rodriguez *et al.* (2003), Gonzalez *et al.* (1999), Escobar *et al.* (1996) and Harneit *et al.* (2005) as summarised in Table 4.2 below.

In this study, a marginally greater final attachment of *A. ferrooxidans* were observed for the chalcopyrite mineral concentrate system relative to the pyrite concentrate mineral system with 97 % and 92 % attained in each respective system. However, attachment to pyrite was more rapidly illustrated by 78% and 50% attachment at 5 minutes respectively. Selective and more rapid attachment of *A. ferrooxidans* to pyrite over chalcopyrite was reported by Rodriguez *et* *al.* (2003) and Harneit *et al.* (2005). Rapid attachment to pyrite was also demonstrated by Gonzalez *et al.* (1999) with the establishment of attachment equilibrium within 30 minutes.

Table 4.2: Summary of the attachment efficiency of *A. ferrooxidans* to pyrite and chalcopyrite concentrates as reported in literature.

Author	**Method**	**Particle size** (µm)	**Attachment to pyrite (%)**	**Attachment to chalcopyrite (%)**	**Time to equilibrium (minutes)**
Atkins *et al.* 1986	Nitrogen measurements	- 45	87	-	-
Norris *et al.* 1988	Bacth shake flasks, microbial protein assays	- 75	7	-	-
Natarajan *et al.* 1992	Batch shake flask, microbial protein assays	63 - 75 600 -1000 4000-4750	60.4 40 25	-	-
Ohmura *et al.* 1993	Batch test tube, direct cell counts	61.7-64.6	24	14	-
Escobar *et al.* 1996	Batch shake flasks, Harrison plating	100 – 150	-	77	120
Gonzalez *et al.* 1999	Batch shake flask, microbial protein	35 -75	*		30
Sampson *et al.* 2000	Batch test tube OD readings	32 – 53	25.9	4.4 – 8	-
Rodriguez *et al.* 2003	Batch shake flasks, direct cell counts	2 – 44 44 – 149	100 -	- 64	1 200
Harneit *et al.* 2005	Batch shake flasks, direct cell counts	50 – 100	85	- 60 – 70	30 60
This study	Batch shake flasks, direct cell counts	10 - 60 10 - 40	92 -	- 97	120 120

The percentage attachment to pyrite for Gonzalez *et al.* (1999) was not cited as this value was reported as a microbial protein per gram ore.

Gonzalez *et al.* (1999) and Natarajan *et al.* (1992) have reported the observation that equilibrium attachment efficiency may be affected by particle size, where the attachment of *A. ferrooxidans* was shown to decrease with increasing particle size. The smaller size fraction used in this study (between 10 and 40 µm), relative to Rodriguez *et al.* (between 44 and 149 µm) and Harneit *et al.* (between 50 and 100 µm) provides an increased surface area available for attachment allowing for higher levels of attachment to be achieved. Variations in the extent of attachment observed could be attributed to the microbial strain used (Harneit *et al.* 2005, Gehrke *et al.* 1998, Gehrke *et al.* 2001, Sampson *et al.* 2000).

The earliest report of selective adhesion by *A. ferrooxidans* to pyrite mineral surfaces over chalcopyrite and quartz was by Ohmura *et al.* (1993). However, maximum attachment efficiency attained as reported by Ohmura *et al.* (1993) was only 25 % to pyrite, 14 % to chalcopyrite and 4.6 % to quartz. The low levels of attachment reported by Ohmura *et al.* (1993) relative to the findings of this study and those cited in literature can not be explained. Similarly, selective attachment of *A. ferrooxidans* to sulfide minerals was reported by

Sampson *et al.* (2000). This group also reported considerably lower levels of attachment of ferrous sulphate grown *A. ferrooxidans* to sulfide minerals (25.9 % and 4.4 % to pyrite and chalcopyrite respectively). However the experimental approach used by Sampson *et al.* (2000) was markedly different to that used in this study as it only provided an estimate of the extent of attachment over a contact period of 10 minutes and was not expected to determine equilibrium attachment and which may explain discrepencies in the results (Table 4.2).

Low levels of attachment of *A. ferrooxidans* to quartz observed in this study were consistent with those reported in literature. Harneit *et al.* (2005) report 18% attachment to quartz after one hour, which subsequently increases to 50 % after 8 hours. Attachment of *A. ferrooxidans* to quartz is within the range observed by Harneit *et al.* (2005). Adhesion of cells to vessel walls was assumed to be negligible thus lower levels of attachment to quartz and low grade chalcopyrite ore can not be attributed to this. This study represents the first report of quantitative assessment of attachment of microorganisms to low grade chalcopyrite containing mineral ores. A slight preference for attachment to low grade chalcopyrite containing mineral ore over quartz was demonstrated. This was likely due to the presence of, and interaction of microbes with, sulfide minerals, namely pyrite (4 % wt) and chalcopyrite (0.2 % wt), in the low-grade ore,

4.4 CONCLUSION

The purpose of the research is to investigate the attachment of microorganisms commonly isolated from heap bioleach environments to sulfide mineral surfaces. Consequently, this project serves to address the need for a further understanding of microbial colonisation in heap leach environments by answering the following key questions: Do bioleach microorganisms exhibit preferential attachment to certain mineral surfaces and if so what influences this?

Rapid, selective attachment by *A. ferrooxidans* to sulfide minerals with preference given to sulfide minerals relative to low-grade chalcopyrite containing mineral ore and quartz was demonstrated. Rapid attachment was observed when contacting microorganisms with the sulfide mineral concentrates. High levels of attachment were attained to sulfide minerals (92 % and 97 % attachment to pyrite and chalcopyrite respectively), with attachment to quartz and low grade chalcopyrite containing mineral ore only reaching 50 % of the maxima reached to sulfide minerals (52 % and 50 % attachment to low grade ore and quartz respectively) summarised in Table 4.1. Further this maximum attachment to low grade chalcopyrite containing mineral ore and gangue was not saturated and decreased to 32 and 23 % respectively. The findings for the sulfide minerals are in accordance with those reported in literature (Atkins *et al.* 1986, Escobar *et al.* 1996, Rodrieguez *et al.* 2003, Harneit *et al.* 2005, Gonzalez *et al.* 1999) with some discrepancies in the maximum attachment reached reported by Ohmura *et al.* (1993) and Sampson *et al.* (2000).

Investigation of attachment reported in the literature makes use of batch agitated or shake flask systems. These approaches do not necessarily provide a reliable representation of attachment in a heap environment. The use of mineral concentrates provides a large surface area and ensures this is well contacted with the planktonic cell population in an aqueous environment. Hydrodynamic conditions experienced in batch agitated systems differ to those encountered in a heap, providing both improved contacting and higher levels of attrition to detach microbes. Shake flask systems present a well-mixed environment which may facilitate more effective particle transport. The likelihood of microorganisms encountering mineral surfaces is increased. This may have resulted in the higher levels of attachment particularly to sulfide minerals than may occur in a heap environment, hence these studies are expected to be of limited value in modelling or predicting adhesion in the heap environment. In contrast, heap operations operate as flow through systems, where microorganisms suspended in an acidic medium are percolated through a heap of low-grade ore and fresh medium is continuously introduced.

The findings of this study, though indicative of selective attachment of *A. ferrooxidans* to sulfide minerals are not expected to represent attachment dynamics adequately in a heap environment. To facilitate process modelling a more representative means of quantitatively investigating attachment relevant to heap bioleaching is required, which is the focus of the next chapter.

Chapter 5: Packed column attachment study

5.1 INTRODUCTION

To date research on microbial attachment in a bioleach context has been conducted using batch agitated shake flask systems which do not adequately represent hydrodynamic conditions and contacting in a bioleach heap. More innovative and representative means of quantitatively investigating attachment need to be used in order to facilitate process modelling. In this approach, three identical glass packed column reactors were used to investigate the propensity of *A. ferrooxidans*, *L. ferriphilum* and *S. metallicus* to attach to four different mineral surfaces, namely: pyrite concentrate, chalcopyrite concentrate, low-grade chalcopyrite containing milled ore sample, and quartz.

Each reactor was loaded with 300 mineral coated beads of 6mm diameter and was operated at ambient temperature as a continuous flow through system. Freshly coated beads were used for every experiment. Feed was introduced from the bottom of the column at a constant rate of 1 ml min^{-1} and the columns operated as saturated systems to ensure consistent exposure of microbes across the mineral surface. The reactors were saturated with 9 K media at a pH of 2.5 (unless otherwise stated), prior to inoculation, whereafter fresh 9 K media (pH 2.5) was introduced continuously. For the inoculation period, a 10 ml or 15 ml volume of a 9 K suspension containing the microorganism of interest was introduced into the column as a pulse.

The effluent stream was fractioned into 15 ml aliquots, the cell concentration enumerated microscopically via direct counting, and the attachment efficiency assessed. The attachment efficiency represents the percentage inoculum remaining in the column at a particular sample point and was represented graphically against time. The pH, redox potential, conductivity and ferrous iron content of eluted samples were measured. An experiment of 285 minutes was conducted to ascertain and validate the time taken for the attainment of an attachment plateau under these experimental conditions. Subsequently, the standard duration of the experiments undertaken was 135 minutes, representing two to three residence times, where the residence time of the column reactor was calculated to be approximately 56 minutes (Appendix C1) with the reactor having a void volume of 55 ml. Results were presented as an average attachment efficiency, representative of triplicate results.

The following assumptions were made:

- entrapment of cells in the interstitial fluid was negligible
- attachment of cells to reactor vessel walls was negligible
- cells used for inoculation of the column were in an actively growing stage
- there was insufficient time for microbial growth or development of firmly attached colonies and biofilms within the reactor under these experimental conditions
- retention of cells within the column was primarily a result of attractive and repulsive forces between mineral and microbial surfaces contributing to the initial attachment stage as explained in Chapter 2 – Section 2.4
- effect of Bostik glue was negligible under these experimental conditions

5.2 AN ASSESSMENT OF THE TIME TAKEN FOR ATTACHMENT TO LEVEL OFF

An experiment to investigate the time taken for attachment of *A. ferrooxidans* to a chalcopyrite mineral concentrate was conducted in a packed-column reactor at pH 1.5. The duration of the experiment totalled 285 minutes which corresponded to approximately five residence times to determine the overall attachment efficiency, the time taken for the onset of attachment and to ascertain how long it took for microbial attachment to level off under these conditions. This study allowed for optimisation and validation of the column attachment approach for subsequent investigations. The results of the experiment are presented in Figure 5.1, where the change in the number of cells that remained in the column given as a percentage of the initial inoculum cell concentration is presented as a function of time. Data were reported as an average of triplicate results. Errors were typically small (less than 5 %) indicative of good reproducibility of the experimental run.

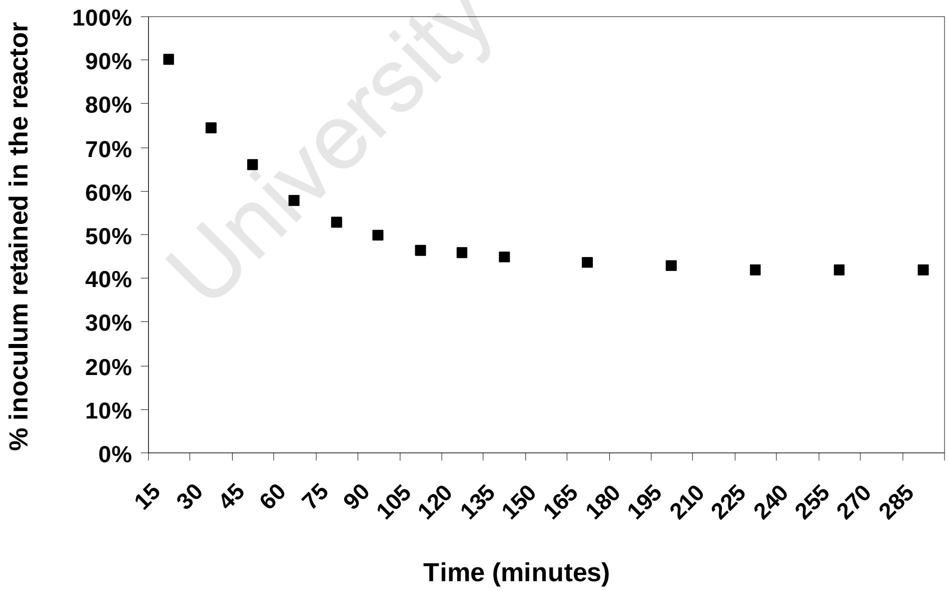

Figure 5.1: Attachment of *A. ferrooxidans* to chalcopyrite mineral concentrate. Cell retention in the column as the experiment progressed is represented as a percentage of the *A. ferrooxidans* inoculum remaining in the column. Samples were taken in 15 ml aliquots with the reactor operated at 1ml min^{-1}, thus the first data point is only represented after 15 minutes.

Upon analysis of in Figure 5.1, an initial decrease in the number of *A. ferrooxidans* cells that remained in the column was evident on elution with fresh 9 K. Thereafter the number of cells remaining in the column approached a constant value asymptotically. After approximately one residence time (approximately 60 minutes), only 58 % of the inoculum remained in the column. The rate of the cells leaving the reactor was observed to be an inverse function of their attachment. After two residence times (120 minutes) the number of cells that remained in the column began to level off. The number of cells retained in the reactor decreased to 46 % after the second residence time, and marginally, to 42 % after three residence times (180 minutes). Following this, no further decrease in the number of cells which remained in the column was evident. After five residence times (at the 285 minute sampling point) 42 % of the inoculum was still retained in the reactor. This final number of cells which remained in the column is referred to as the attachment efficiency.

Table 5.1: Summary of the attachment efficiency and the cell attachment per available surface area of ore-free cultured *A. ferrooxidans* to a chalcopyrite mineral concentrate, after a 285 minute period in a saturated, continuous flow through, packed column reactor system. For inoculation of the column, 10 ml at a cell concentration of 1 x 10^8 cells ml^{-1} was used.

Microorganism	Residence time	% Inoculum retained	Cells retained per available surface area (cells m^{-2})
A. ferrooxidans	1 (60 minutes)	58 ± 0.7	1.8 x 10^{10} ± 0.016 x 10^{10}
	2 (120 minutes)	46 ± 0.1	1.5 x 10^{10} ± 0.0037 x 10^{10}
	135 minutes	45 ± 0.5	1.4 x 10^{10} ± 0.014 x 10^{10}
Final attachment efficiency	(285 minutes)	42 ± 1	1.3 x 10^{10} ± 0.013 x 10^{10}

The attachment efficiency achieved per unit surface area is summarised in Table 5.1 as is the cell retention represented as a percentage of the inoculum retained in the column reactor. The inoculum retained after 135 and 285 minutes was 45 ± 0.5 % and 42 ± 1 %. These percentages equate to 1.4 x 10^{10} ± 2.3 x 10^8 and 1.3 x 10^{10} ± 4.3 x 10^8 cells m^{-2} attached at each respective sampling point. These results illustrate that attachment levels off after two residence times as 93 % of the cells retained per meter square after 135 minutes are still retained after 285 minutes. Subsequent experiments were conducted for 135 minutes only as this was demonstrated to be an adequate amount of time for cells to interact with the mineral surface and for these reversible interactions to be maintained under these experimental conditions.

5.3 INVESTIGATION OF THE EFFECT OF VARYING CULTURE CONDITIONS OF THE ATTACHMENT OF MESOPHILES TO CHALCOPYRITE CONCENTRATE

Experiments to investigate the effect of varying culture conditions on the propensity of *A. ferrooxidans* and *L. ferriphilum* to attach to chalcopyrite mineral concentrate systems were conducted. Microorganisms were cultured either in the presence (sulfide mineral-adapted or "MA") or absence (ore-free or "OF") of solid sulfide mineral particles. The experiments were conducted as described previously for a period of 135 minutes at a pH of 2.5. Data was reported as an average of triplicate results. The error bars represent standard deviations in the results which were typically small (mostly less than 5 %). Results of experiments conducted using ore-free cultured mesophiles are presented in Section 5.3.1, followed by the results observed for mineral-adapted cultures in Section 5.3.2. The effect of solution chemistry is examined in Section 5.3.3, with a discussion of results provided in Section 5.3.4.

5.3.1 Attachment of *A. ferrooxidans* and *L. ferriphilum*, cultured in the absence of sulfide mineral concentrate, to a chalcopyrite mineral concentrate: Results

For the experiments conducted with ore-free *A. ferrooxidans* and *L. ferriphilum* inoculum, the cell number remaining in the column with time and thereby eluent volume is presented as a percentage of the inoculum cell concentration in Figure 5.2. The attachment efficiency was calculated as a function of the surface area available for attachment (cells attached m^{-2}) and the data contrasted with the cell retention in the column as a percentage of inoculum concentration used in Table 5.2.

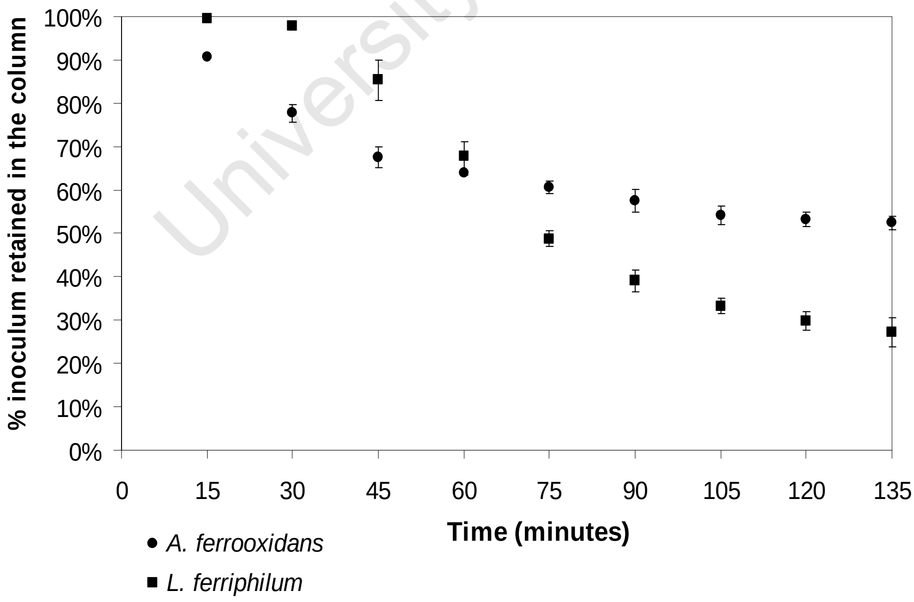

Figure 5.2: Attachment of *L. ferriphilum* and *A. ferrooxidans* cultured in the absence of ore (OF) to a chalcopyrite mineral concentrate packed column reactor system. The change in the number of cells that remained in the column as the experiment progressed is represented as a percentage of the inoculum remaining in the column. Samples were taken in 15 ml aliquots with the reactor operated at 1ml min^{-1}, thus the first data points are only represented after 15 minutes.

From Figure 5.2 it was evident that the initial decrease in cell retention was less rapid relative for *L. ferriphilum* relative to *A. ferrooxidans* for the first 30 minutes. The cell retention between the 15 minute (first) and 30 minute (second) sample point decreased from 100 ± 0.2 to 98 ± 1.0 % for *L. ferriphilum* and 91± 0.2 to 78 ± 2.0 % for *A. ferrooxidans.* Between the 30 minute sample point and the first residence time (60 minute sample point) the decrease in the cell retention is more rapid for *L. ferriphilum* relative to *A. ferrooxidans* with cell retention decreasing from 98 ± 1.0 to 68 ± 3.4 % and 78 ± 2.0 to 64 ± 0.5 % for each microorganism respectively.

Table 5.2: Comparison of the level of cell retention and attachment efficiencies of *L. ferriphilum* and *A. ferrooxidans,* cultured in the absence or presence of sulfide mineral concentrate, in a packed column reactor charged with chalcopyrite mineral coated beads. Retention of cells in the reactor as the experiment progressed is presented as a percentage of the inoculum added and per available surface area for attachment.

Microorganism	**Residence time**	***A. ferrooxidans***		***L. ferriphilum***	
Ore-free culture		% Inoculum retained	(cells m^{-2}) ± 1 x 10^9	% Inoculum retained	(cells m^{-2}) ± 3 x 10^9
	1	64 ± 0.5	3.2 x 10^{10}	68 ± 3.4	3.7 x 10^{10}
	2	53 ± 2.0	2.9 x 10^{10}	30 ± 2.2	1.7 x 10^{10}
Overall attachment efficiency		52 ± 1.5	2.8 x10^{10}	27 ± 3.4	1.5 x10^{10}

From the results presented in Table 5.2, the level of cell retention observed at the first residence time was similar for *A. ferrooxidans* and *L. ferriphilum* with a 64 ± 0.5 and 68 ± 3.4 % inoculum retention achieved respectively. This equated to 3.7 x 10^{10} ± 3 x 10^9 and 3.2 x 10^{10} ± 1 x 10^9 cells m^{-2} for *L. ferriphilum* and *A. ferrooxidans* respectively. Attachment levelled off after two residence times for both microorganisms. Higher overall attachment efficiencies were observed for *A. ferrooxidans* relative to *L. ferriphilum* with 52 ± 1.5 and 27 ± 3.4 % inoculum retained in the column equating to an attachment of 2.8 x10^{10} ± 1 x 10^9 and 1.5 x10^{10} ± 3 x 10^9 cells m^{-2} respectively (Table 5.2). The observation of an attachment “plateau” for both microorganisms may lead one to infer that attachment must be selective, since the effects of fluid flow had to be overcome for this to occur. Had this not been the case, complete wash out of cells from the column would have been observed.

5.3.2 Attachment of *A. ferrooxidans* and *L. ferriphilum*, cultured in the presence of sulfide mineral concentrate, to a chalcopyrite mineral concentrate: Results

Results observed for the attachment of *A. ferrooxidans* and *L. ferriphilum* to a chalcopyrite mineral concentrate as a function of pre-culture conditions is presented in Figure 5.3. Upon interrogation of the results obtained for mineral-adapted *A. ferrooxidans*, a gradual decrease in the number of cells that remained in the column was observed with time with 69 ± 1.2 % of the inoculum retained in the vessel after one residence time. The cell retention decreased further, and then levelled off after two residence times with 64 ± 1.2 % cell retention achieved. The overall attachment efficiency achieved was 63 ± 1.1 %. The trend observed for the mineral-adapted *A. ferrooxidans* culture was similar to that of the ore-free culture. However, a consistently greater level of cell retention was achieved in the mineral-adapted *A. ferrooxidans* study relative to the ore-free *A. ferrooxidans* study, illustrated in Figure 5.3.

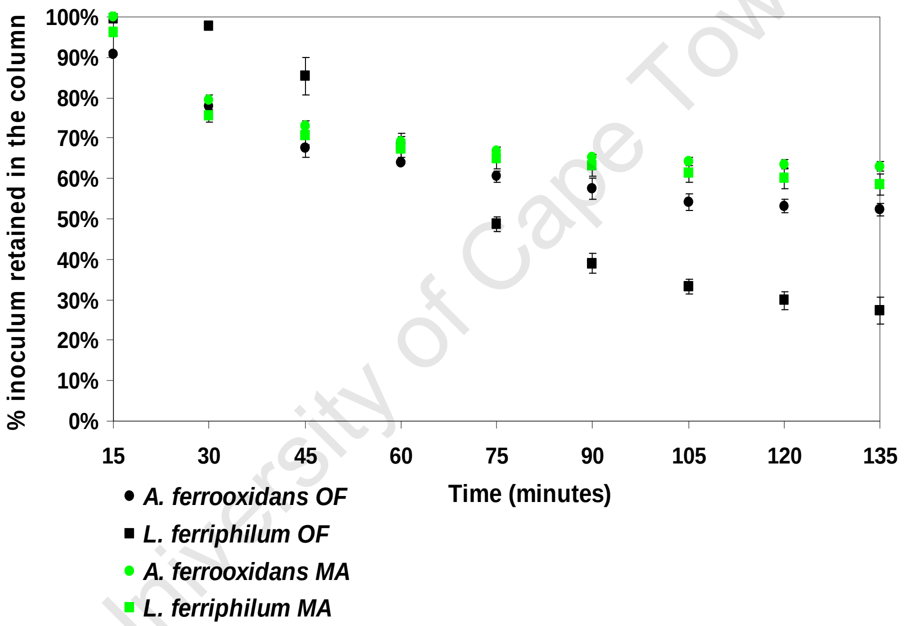

Figure 5.3: Attachment of *L. ferriphilum* and *A. ferrooxidans* cultured either in the absence of ore (OF) or adapted to sulfide mineral systems (MA), to chalcopyrite mineral concentrate in a packed column reactor system.

These data are presented in Table 5.3 as a percentage of the inoculum and as a function of the available surface area. After the first residence time 69 ± 1.2 % of the inoculum was still retained in the column for the mineral-adapted culture, which equated to 6.4 $x10^{10}$ ± 3 x 10^{9} cells m^{-2} attached. For the ore-free culture 64 ± 0.5 % of the inoculum was retained in the vessel after the first residence time which equated to only 3.2 x 10^{10} ± 1 x 10^{9} cells m^{-2} attached. This trend was also apparent when comparing cell retention after the second residence time as well as overall attachment efficiencies achieved of the ore-free relative to the mineral-adapted *A. ferrooxidans* culture, seen in Table 5.3. The larger differences in retention per unit surface unit surface area are introduced owing to variation in the inoculation size.

Table 5.3: *A. ferrooxidans* and *L. ferriphilum* cell retention in column reactors charged with chalcopyrite mineral coated beads is presented as a percentage of the inoculum added. The corresponding level of attachment per available surface area is also presented.

Growth history	Residence time	*A. ferrooxidans*		*L. ferriphilum*	
Ore-free culture		% Inoculum retained	(cells m^{-2}) $\pm 1 \times 10^{9}$	% Inoculum retained	(cells m^{-2}) $\pm 3 \times 10^{9}$
	1	64 ± 0.5	3.2×10^{10}	68 ± 3.4	3.7×10^{10}
	2	53 ± 2.0	2.9×10^{10}	30 ± 2.2	1.7×10^{10}
Overall attachment efficiency		52 ± 1.5	2.8×10^{10}	27 ± 3.4	1.5×10^{10}
Mineral-adapted		% Inoculum retained	(cells m^{-2}) $\pm 3 \times 10^{9}$	% Inoculum retained	(cells m^{-2}) $\pm 5 \times 10^{9}$
	1	69 ± 1.2	6.4×10^{10}	67 ± 2.3	6.4×10^{10}
	2	64 ± 1.2	5.9×10^{10}	60 ± 2.6	5.7×10^{10}
Overall attachment efficiency		63 ± 1.1	5.9×10^{10}	58 ± 2.6	5.6×10^{10}

Upon analysis of Figure 5.3 and Table 5.3 for the mineral-adapted *L. ferriphilum* culture, a slow decrease in the number of cells that were retained in the column as the experiment progressed was observed with 67 ± 2.3 % of the inoculum retained in the column after one residence time. Attachment levelled off after two residence times, with a 60 ± 2.6 % inoculum retention achieved. This level of cell retention decreased only marginally with an overall attachment efficiency of 58 ± 2.6 % at 135 minutes.

The trend observed for mineral-adapted *L. ferriphilum* differed from the ore-free grown *L. ferriphilum* culture. For *L. ferriphilum* cultured in the absence of sulfide mineral concentrate, the initial decrease in cell retention was less subtantial relative to the mineral-adapted culture. The decrease in cells retained in the reactor between the 15 minute (first) and 30 minute (second) sample point was from 100 ± 0.2 to 98 ± 1.0 % and 96 ± 0.6 to 75 ± 1.6 % for ore-free cultured and mineral-adapted *L. ferriphilum* respectively.

The decrease in level of cells that remained in the column became more significant for the ore-free culture relative to the mineral-adapted culture between the 30 minute sample point and the first residence time with the proportion of cells retained after the first residence time being similar. Cell retention decreased from 98 ± 1.0 to 68 ± 3.4 % and 75 ± 1.6 to 67 ± 2.3 % for ore-free and mineral-adapted cultures respectively. Cell retention as a percentage of inoculum equated to 3.7 x 10^{10} ± 3 x 10^9 and 6.4 x10^{10} ± 5 x 10^9 cells attached m^{-2} for ore-free and mineral-adapted cultures respectively, seen in Table 5.3.

The ore-free *L. ferriphilum* culture continued to decrease substantially relative to the mineral-adapted cultures with a 30 ± 2.2 and a 60 ± 2.6 % inoculum retention achieved after the second residence time respectively. Attachment plateaus for both ore-free and mineral-adapted cultures after the second residence time with a higher overall attachment efficiency of 58 ± 2.6 % achieved for mineral-adapted cultures relative to the ore-free cultures where an attachment efficiency of 27 ± 3.4 % was observed. These values equate to 5.6 x10^{10} ± 5 x 10^9 and 1.5 x10^{10} ± 3 x 10^9 cells attached m^{-2} for mineral-adapted cultures and ore-free cultures respectively (Table 5.3). Higher overall attachment efficiencies for mineral-adapted culture relative to ore-free culture may suggest that ore-free *L. ferriphilum* has a lesser propensity for attachment to chalcopyrite mineral concentrate than mineral-adapted *L. ferriphilum* culture.

The trend observed for attachment of mineral-adapted *L. ferriphilum* was similar to that observed for *A. ferrooxidans* cultured in the presence of sulfide mineral concentrate, with comparable cell retention and level of attachment per available surface area achieved after the first and second residence time (Table 5.3). The overall attachment achieved per available surface area was marginally higher for mineral-adapted *A. ferrooxidans* relative to mineral-adapted *L. ferriphilum* were 5.9 x10^{10} ± 3 x 10^9 and 5.6 x10^{10} ± 5 x 10^9 cells attached m^{-2} respectively. The results illustrated that mineral-adapted *L. ferriphilum* and *A. ferrooxidans* exhibit similar attachment trends and attachment efficiencies. Mineral-adapted cultures exhibited greater affinities for attachment to chalcopyrite relative to ore-free cultures.

5.3.3 The effect of solution chemistry on the attachment of mesophiles to chalcopyrite mineral concentrate

The pH, redox potential and conductivity of the standard solution feeds were pH 2.57, 394 mV and 5.01 mS. Trends in these characteristics with passage through the columns are given in Figure 5.4. The pH and conductivity remained within range of the standard solutions. The average pH, redox potential and conductivity of the solution that exited the three columns ranged from pH 2.46 to pH 2.61, 421 mV to 473 mV and 5.21 mS to 5.67 mS. Although redox potential increased, it was assumed, given the time span of the experiment that the increase in redox potential was not a result of microbially assisted leaching. The pH and conductivity remained within range of the standard solutions.It was assumed that the trends in attachment observed were not due to a change in solution chemistry, and that no microbially assisted leaching occurred during the span of the experiment.

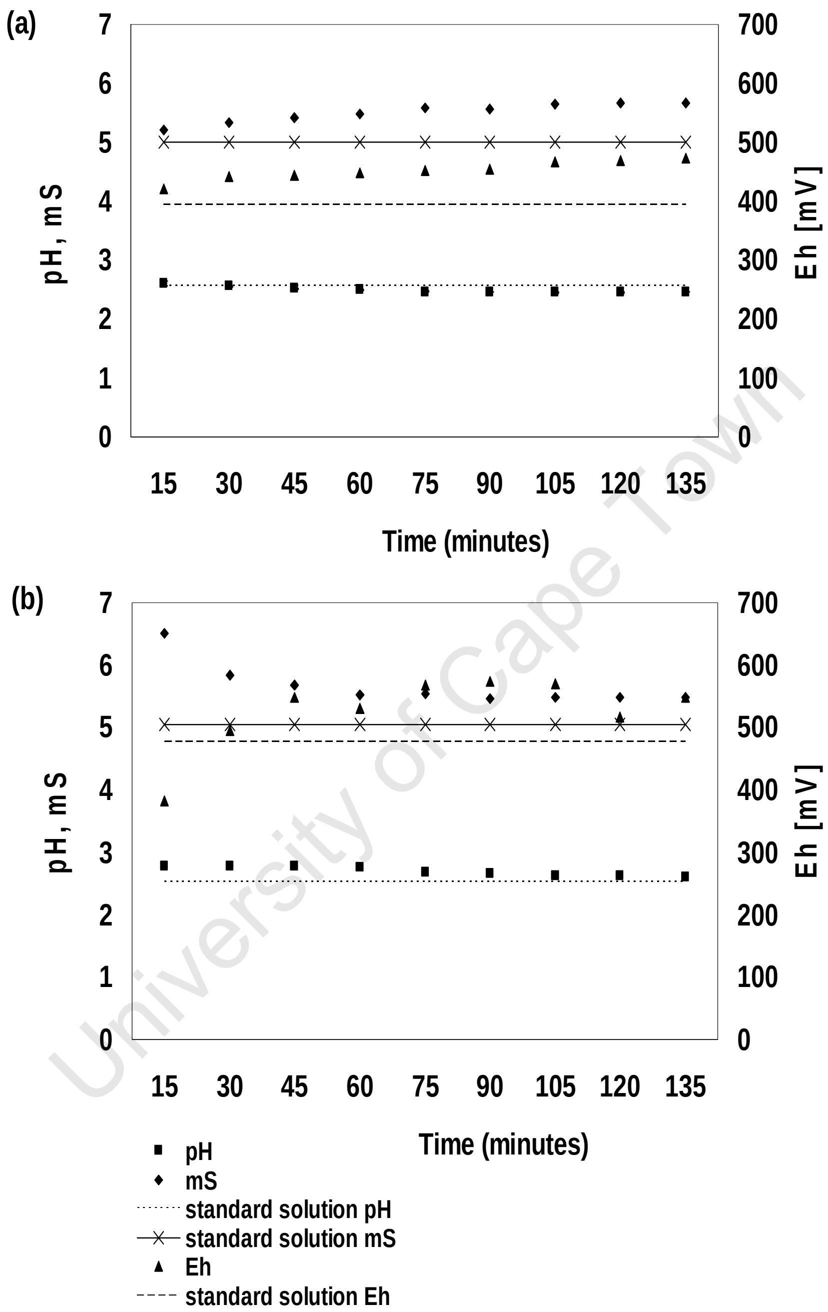

Figure 5.4: Trends in pH, redox potential and conductivity of the standard solution feeds with passage through the columns are given for the attachment of **(a)** *A. ferrooxidans* and **(b)** *L. ferriphilum* to chalcopyrite mineral concentrate in a packed column reactor.

For the experiment conducted at pH 1.5 where the irrigation was extended to determine how long attachment took to level off under these experimental conditions; the eluent pH ranged from a pH of 1.65 to pH 1.74 throughout the duration of the experiment. The redox potential ranged from 373 mV to 415 mV which was comparable to the standard feed solution. The conductivity of the standard feed solution at pH 1.5 was 23.5 mS which was 4 times greater than that of the pH 2.5 experiment. The conductivity of the exiting solution ranged from 18.3 mS to 23.5 mS which was within the range of the standard feed solution. Upon decreasing the pH from 2.5 to 1.5, a 7 % decrease in cell retention was observed after 135 minutes (cell retention decreased from 52 % to 45 % experiments conducted at pH 2.5 and pH 1.5 respectively).

5.3.4 Attachment of mesophiles to chalcopyrite mineral concentrate: Discussion

The attachment and subsequent colonisation of microorganisms to solid surfaces is presented as a four-stage process (van Loosdrecht *et al.* 1990). Initial adsorption requires transport of the organisms to the surface of the solid followed by a transient reversible stage. This is succeeded by a more permanent interaction, which results in firm attachment. Subsequently, colonisation of the surface and the formation of biofilms occur (Section 2.4). It was presumed that the period of the experiment did not permit significant growth or development of firmly attached colonies or biofilms. Therefore, the adsorption trends observed are discussed with respect to forces responsible for the initial attachment stage.

The establishment of an attachment plateau or levelling off of attachment was used to estimate the attachment efficiency. The levelling off of attachment observed for all studies provides evidence of selective attachment as cells were not observed to wash out of the reactor and the forces imposed by continuous fluid flow through the reactor had to be overcome for this plateau effect to be achieved. The fluid flow regime through the column was determined by calculating the dimensionless Reynolds number (see Appendix G1). A Reynolds number less than 2100 indicates fluid flow within the laminar flow regime. Conversely, a Reynolds number greater than 2100 is indicative of turbulent fluid flow. The Fluid flow under the conditions of this experiment fell within the laminar flow regime, indicated by a Reynolds number of 0.266884 (see Appendix G).

Table 5.4: Attachment of mesophilic microorganisms to chalcopyrite reported in literature and contrasted with the results of this study.

Author	**Method**	**Microorganism**	**Particle size** (μm)	**Attachment efficiency (%)**	**Time to attachment plateau**
Ohmura *et al.* 1993	Batch test tube, direct cell counts	*A. ferrooxidans*	61.7-64.6	14	-
Escobar *et al.* 1996	Batch shake flasks, Harrison plating	*A. ferrooxidans*	100 – 150	77	120
Sampson *et al.* 2000	Batch test tube OD readings	*A. ferrooxidans* (OF)	32 – 53	4.4 – 8	-
		A. ferrooxidans (MA)		13.6	
Rodriguez *et al.* 2003	Batch shake flasks, direct cell counts	Mixed culture (*Acidithiobacillus, Leptospirillum sp*)	44 – 149	64	200
Harneit *et al.* 2005	Batch shake flasks, direct cell counts	*A. ferrooxidans*	50 – 100	60 – 70	60
This study	Packed bed reactor, direct cell counts	*A. ferrooxidans* (OF)	(n.a)	52 ± 1.5	120
		A. ferrooxidans (MA)		63 ± 1.1	120
		L. ferriphilum (OF)	(n.a.)	27 ± 3.4	120
		L. ferriphilum (MA)		58 ± 2.6	120

The attachment of mesophilic microorganisms to chalcopyrite reported in literature to date is summarised in Table 5.4. Similar trends of attachment were observed for the attachment of *A. ferrooxidans* and *L. ferriphilum* to chalcopyrite, with the exception of the ore-free grown *L. ferriphilum* culture. The levels of attachment to chalcopyrite achieved for *A. ferrooxidans* (OF), *A. ferrooxidans* (MA) and *L. ferriphilum* (MA) in this study range between 52 and 63 %. Since a chalcopyrite concentrate was used, higher levels of attachment were expected from previous shake flask studies. The results of this study fall within the lower range of that observed for attachment of mesophilic microorganisms to chalcopyrite by Harneit *et al.*, Rodriguez *et al.*, and Escobar *et al.*, where attachment efficiencies of 60 and 77 % were observed in batch shake flask studies (Table 5.5). Slightly lower levels of attachment observed in this study relative to literature reports are expected to be due to differences in experimental approach. The column approach operated as a saturated continuous flow through system. Higher levels of attachment in literature relative to this study may be due to improved contacting in batch agitated systems. Despite the difference in experimental approach, the hypothesis of 135 minutes (two residence times) being sufficient time for attachment to level off for mesophiles attaching to chalcopyrite was consistent with literature reports by Rodriguez et al. (2003) and Escobar et al. (1996).

The attachment efficiency observed for *L. ferriphilum* (OF) was lower than literature reports, with the trend of attachment observed to be different to that observed for the other mesophilic microorganisms investigated. Based on discussions of initial adsorption in Section 2.4, this observation suggests a dependence of surface properties on culture conditions. For experiments where variations in the inoculum concentration were apparent, the attachment efficiency was calculated as a function of the cell attachment achieved per available surface area. The results are summarised in Table 5.5. Results observed for the experiment conducted for the extended period (285 minutes) as well as subsequent experiments conducted for 135 minutes are summarised and contrasted. The saturation cell number, representing the maximum number of cells accommodated by the available surface area, was calculated to be 4.5 x 10^{10} cells. This saturation number was never exceeded; with inoculum concentrations used provided a maximum of only 7 % coverage of the available surface area. Thus it was deduced that variations in inoculum concentration did not greatly influence the results observed. The effects of fluid flow and contacting may have played a more substantial role in affecting initial adhesion interactions and thus subsequent attachment efficiencies under these experimental conditions.

Table 5.5: Summary of the inoculum concentrations used for attachment of mesophiles cultured in the absence of (OF) and adapted to (MA) sulfide mineral concentrate, to chalcopyrite. The corresponding attachment efficiencies are presented as a percentage of the inoculum and per surface area available for attachment.

Microorganism	***A. ferrooxidans***			***L. ferriphilum***	
Growth history	**OF (285) pH 1.5**	**OF (135) pH 2.5**	**MA (135) pH 2.5**	**OF (135) pH 2.5**	**MA (135) pH 2.5**
Inoculum concentration (cells ml^{-1})	1 x 10^8	2.1 x 10^8 ± 1.9 x 10^7	3.3 x 10^8 ± 2.7 x 10^7	1.9 x 10^8 ± 3.3 x 10^7	3.2 x 10^8 ± 2.4 x 10^6
Maximum coverage of surface area available for attachment provided by inoculum (%)	2	4	7	4	7
Attachment efficiency (%)	45 ± 1.5	52 ± 1.5	63 ± 1.1	27 ± 3.4	58 ± 2.6
Attachment efficiency (cells m^{-2})	2.1 x 10^8 ± 1.9 x 10^7	2.8 x 10^{10} ± 1 x 10^9	5.9 x 10^8 ± 3 x 10^9	1.5 x 10^{10} ± 3 x 10^9	5.6 x 10^{10} ± 5 x 10^9

The effects of fluid flow can not be ignored especially when investigating initial attachment in a flow through system. Contacting plays a substantial role in influencing initial adhesion interactions (and thus final attachment efficiencies in this experimental setup) since the first stage in initial adhesion mechanism requires transport of the microorganism to the mineral surface for initial adhesion surface interactions to occur (transient electrostatic and hydrophobic interactions).

Due to the consistency of the extent of the attachment to chalcopyrite mineral concentrate observed for mesophilic microorganisms under the respective culture conditions, it may be inferred that the mechanisms involved in the adhesion interactions are the same and that *A. ferrooxidans* and *L. ferriphilum* display similar affinities for attachment to chalcopyrite mineral concentrate under these experimental conditions. This is supported by a statistical interrogation of the results observed for mineral-adapted cultures. The variance of the attachment efficiencies observed for *A. ferrooxidans* and *L. ferriphilum* was analysed using the ANOVA statistical function in Excel (see Appendix D for full statistical analysis and explanation). Since the F ratio of 2.10 was found to be less that the F critical value of 3.89, it was determined with 95 % confidence that there was no statistically significant difference between the extent of the attachment observed for *A. ferrooxidans* and *L. ferriphilum* to chalcopyrite. Hence, there is statistically significant evidence to suggest, based on the variance of the extents of the attachment efficiencies observed, that the attachment behaviour of these two microorganisms under each respective culture conditioning to chalcopyrite mineral concentrate was similar.

Solution chemistry was considered and results observed illustrated that the attachment observed was not due to a change in solution chemistry as pH, redox potential and conductivity remained within range of the standard solution.

For *L. ferriphilum* and *A. ferrooxidans* cultured under sulfide mineral-adapted conditions enhanced attachment efficiencies were evident relative to ore-free cultures. Final attachment efficiencies achieved were 27 ± 3.4 and 52 ± 1.5 % for ore-free cultured *L. ferriphilum* and *A. ferrooxidans* respectively, with 58 ± 2.6 and 63 ± 1.1% achieved for the same cultures grown in the presence of sulfide minerals respectively. From the summary in Table 5.5., Sampson *et al.* reported chalcopyrite grown *A. ferrooxidans* achieved greater attachment efficiencies relative to ferrous grown cultures with the attachment increasing from 4.4 – 8 % to 13.6 %. The findings of Sampson *et al.* corroborate the findings of this study, namely that growth on sulfide minerals enhances subsequent efficiency relative to growth on ferrous iron. The effect of growth history on surface properties and subsequent attachment trends is discussed fully in Section 5.5.

5.4 INVESTIGATION OF THE EFFECT OF VARYING CULTURE CONDITIONS ON THE ATTACHMENT OF MESOPHILES TO A PYRITE MINERAL CONCENTRATE

Experiments to investigate the effect of varying culture conditions on the propensity of *A. ferrooxidans* and *L. ferriphilum* to attach to a pyrite mineral concentrate system were conducted. The experiments were conducted and data reported as described previously. The change in cell retention in the column on continuous elution is presented in Figure 5.6 and Table 5.6.

5.4.1 Attachment of *A. ferrooxidans* to pyrite mineral concentrate system : Results

Upon examination of Figure 5.4, a similar trend was evident for *A. ferrooxidans* cultured in the absence of, and adapted to, sulfide mineral ore. There was a slow decrease in the number of cells retained in the column as the experiment progressed, with a levelling off of attachment after two residence times. The initial level of cells retained in the vessel was markedly higher for the mineral-adapted culture relative to the ore-free grown culture, with 82 ± 2.6 and 63 ± 1.1 % of the inoculum retained after the first residence time for mineral-adapted and ore-free cultures respectively (Table 5.6).

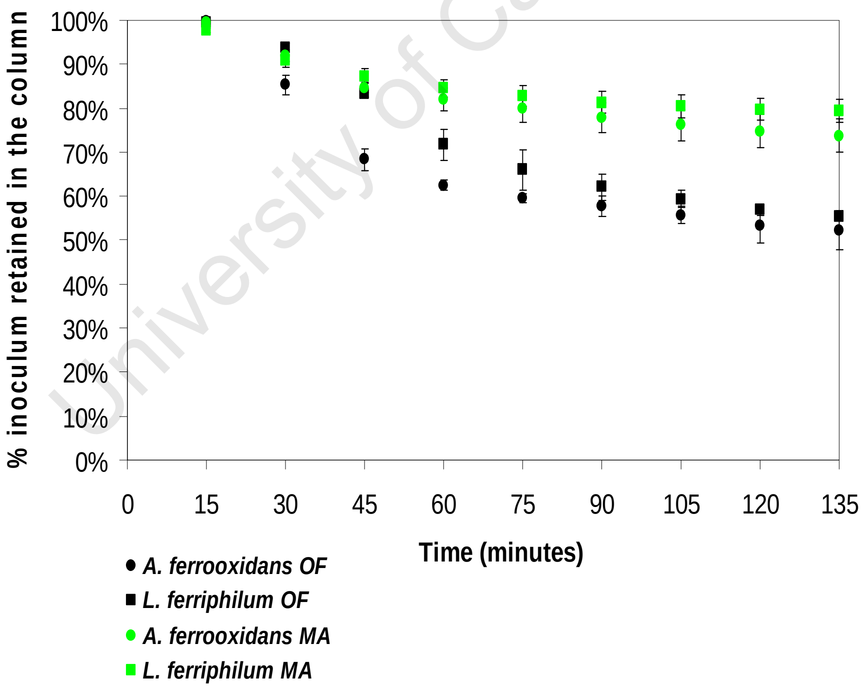

Figure 5.5: Attachment of *L. ferriphilum* and *A. ferrooxidans* cultured either in the absence of ore (OF) or adapted to sulfide mineral systems (MA), to a pyrite mineral concentrate in a packed column reactor system. The change in the number of cells that remained in the column as the experiment progressed is represented as a percentage of the inoculum remaining in the column. Samples were taken in 15 ml aliquots with the reactor operated at 1ml min^{-1}, thus the first data points are only represented after 15 minutes.

The retention of cells in the reactor then decreased to 75 ± 3.7 and 53 ± 3.9 % after the second residence time, which equated to 7.7 $x10^{10}$ ± 1 x 10^{10} and 4.6 x 10^{10} ± 6 x 10^{9} cells m^{-2} for mineral-adapted and ore-free cultures respectively (Table 5.6). Overall attachment efficiencies observed for the mineral-adapted culture was greater relative to the ore-free grown culture, with efficiencies of 74 ± 3.8 and 52 ± 4.3 % attained respectively. This equated to an attachment of 7.5 $x10^{10}$ ± 1 x 10^{10} and 4.5 $x10^{10}$ ± 6 x 10^{9} cells m^{-2} for mineral-adapted and ore-free cultured *A. ferrooxidans* respectively. The consistently greater levels of cell retention in the reactor, as well as higher overall attachment efficiencies observed for mineral-adapted cultures relative to ore-free cultures infers that mineral-adapted cultures exhibit an enhanced affinity for attachment to pyrite mineral concentrate and that growth history affects subsequent attachment preferences of mesophiles to sulfide minerals.

Table 5.6: Comparison of the level of cell retention and the final attachment efficiencies of *L. ferriphilum* and *A. ferrooxidans,* cultured in the absence or presence of sulfide mineral concentrate, in a packed column reactor charged with pyrite mineral coated beads. Retention of cells in the reactor as the experiment progressed is presented as a percentage of the inoculum added and per available surface area for attachment.

Growth history	**Residence time**	***A. ferrooxidans***		***L. ferriphilum***	
Ore-free culture		% Inoculum retained	(cells m^{-2}) ± 6 x 10^{9}	% Inoculum retained	(cells m^{-2}) ± 7 x 10^{9}
	1	63 ± 1.1	5.4 x 10^{10}	72 ± 3.6	2.9 x 10^{10}
	2	53 ± 3.9	4.6 x 10^{10}	57 ± 1.2	2.4 x 10^{10}
Overall attachment efficiency		52 ± 4.3	4.5 $x10^{10}$	55 ± 1.0	2.3 $x10^{10}$
Mineral-adapted		% Inoculum retained	(cells m^{-2}) ± 1 x 10^{10}	% Inoculum retained	(cells m^{-2}) ± 3 x 10^{8}
	1	82 ± 2.6	8.4 $x10^{10}$	85 ± 2.0	8.0 $x10^{10}$
	2	75 ± 3.7	7.7 $x10^{10}$	80 ± 2.5	7.5 $x10^{10}$
Overall attachment efficiency		74 ± 3.8	7.5 $x10^{10}$	79 ± 2.6	7.5 $x10^{10}$

5.4.2 Attachment of *L. ferriphilum* to pyrite mineral concentrate

From Table 5.6 and Figure 5.4, the level of cell retention for *L. ferriphilum* was observed to be more significant for the mineral-adapted culture relative to the ore-free grown culture, with 85 ± 2.0 and 72 ± 3.6 % of the inoculum retained after the first residence time for mineral-adapted and ore-free cultures respectively. Cell retention decreased to 80 ± 2.5 % after the second residence time and 79 ± 2.6 % overall for the mineral-adapted culture. A more substantial decrease was evident for the ore-free culture with a 57 ± 1.2% inoculum retention after the second residence time and 55 ± 1.0 % overall. Attachment efficiency occurred more rapidly for the mineral-adapted culture relative to the ore-free culture with a decrease in cell retention between the first and second residence time of 5 and 15 %.

The attachment trend to pyrite observed for *L. ferriphilum* was the same as that observed for *A. ferrooxidans*: a decrease in the number of cells retained in the reactor was evident as an increasing amount of fluid passed through the reactor with time. Greater attachment efficiency was observed for mineral-adapted cultures relative to ore-free cultures. An attachment plateau was also approached more rapidly for mineral-adapted cultures relative to ore-free cultures. The initial cell retention was observed to be higher for *L. ferriphilum* relative to *A. ferrooxidans*, with 72 ± 3.6 and 85 ± 2.0 % achieved for ore-free and mineral-adapted *L. ferriphilum*, and 63 ± 1.1 and 82 ± 2.6 % reached for ore-free and mineral-adapted *A. ferrooxidans* after the first residence time respectively (Table 5.4). The overall attachment efficiency was observed to be marginally higher for *L. ferriphilum* relative to *A. ferrooxidans*, with 55 ± 1.0 and 79 ± 2.6 % reached for ore-free and mineral-adapted *L. ferriphilum* and 52 ± 4.3 and 74 ± 3.8 % reached for ore-free and mineral-adapted *A. ferrooxidans* respectively. The observation that both mesophilic microorganisms exhibit a similar trend of attachment and attachment efficiency, for the pyrite mineral system, suggests that the mechanism involved in attachment may be similar under these experimental conditions. Growth history affected subsequent attachment behaviour, specifically, growth on pyrite mineral concentrate enhanced attachment to pyrite mineral concentrate (mineral adaptation was carried out using a pyrite mineral concentrate).

5.4.3 The effect of solution chemistry on the attachment of mesophiles to pyrite mineral concentrate

The pH, redox potential and conductivity of the standard solution feeds were pH 2.48, 371 mV and 5.41 mS. For the pyrite mineral system, the pH of the solution exiting the column was substantially higher than the suspending 9 K medium for both the *A. ferrooxidans* and *L. ferriphilum* experiments. The highest pH of 6.12 and 4.24 was measured for the first sample exiting the column for the *A. ferrooxidans* and *L. ferriphilum*–pyrite mineral systems respectively, whereafter the pH decreased to an equilibrium pH of approximately pH 3.6 for both systems. The increase in pH may be due to abiotic pyrite dissolution or gangue dissolution as the column was saturated with 9 K medium at pH 2.5 the day prior to the experiment. The redox potential and conductivity remained within range of the standard solution; with measurements between 287 and 356 mV, and 4.6 and 4.8 mS observed for the *L. ferriphilum* system, and 287 and 356 mV, and 4.5 and 4.9 mS observed for the *A. ferrooxidans* system. See data appended in Appendix C.

5.4.4 Attachment of mesophiles to pyrite mineral concentrate: Discussion

For the mineral-mesophilic microbial systems using pyrite, the number of cells retained in the packed bed reactor decreased with time and then levelled off after two residence times. Attachment was observed to be selective as attachment levelled off, high levels of attachment were observed and wash out of cells from the reactor was not evident. Attachment efficiencies observed ranged from 52 ± 4.3 to 74 ± 3.8 % for *A. ferrooxidans* and 55 ± 1.0 and 79 ± 2.6 % *L. ferriphilum* with the extent of attachment dependent on the growth history of the respective microorganism. The variance of the attachment efficiencies for *A. ferrooxidans* and *L. ferriphilum* was analysed using the ANOVA statistical function in Excel (Appendix D). Since the F ratio of 2.10 was found to be less that the F critical value of 3.89, it was determined with 95 % confidence that there was no statistically significant difference between the extent of the attachment to pyrite of *A. ferrooxidans* and *L. ferriphilum* prepared under the same growth conditions.

Several authors have demonstrated selective attachment of mesophilic microorganisms to pyrite. The attachment efficiencies previously published are summarised in Table 5.7 and compared with the results observed in this study. The growth history of the microorganism and method of determining attachment are also noted in Table 5.7.

The attachment efficiencies observed for mineral-adapted mesophiles in this study were slightly lower but within the range reported in literature by Atkins *et al.* (1986), Norris *et al.* (1988), Rodriguez *et al.* (2003) and Harneit *et al.* (2005) (Table 5.7). On comparison of attachment efficiencies, differences in experimental approach are noted. These are expected to influence both the extent of attachment and the time taken for attachment to level off. In this study, the time taken for attachment to plateau was observed to be roughly 120 minutes which was not consistent with pyrite-mesophilic systems reported in literature by Harneit *et al.* (2005) and Rodriguez *et al.* (2003) where attachment equilibrium was reached after 30 and 1 minute respectively. However, the latter were not regularly contacted with fresh medium. The "instantaneous" attainment of equilibrium attachment reported in literature is indicative of the microorganisms under investigation exhibiting a high affinity for pyrite mineral concentrate, which has been demonstrated in this study through the non-occurrence of washout despite constant fluid flow.

Table 5.7: Published levels of attachment of mesophilic microorganisms to pyrite, compared with the current study.

Author	**Method**	**Microorganism**	**Particle size (µm)**	**Attachment efficiency (%)**	**Time to equilibrium (minutes)**
Atkins *et al.* 1986	Nitrogen measurements	*A. ferrooxidans*	< 45	87	-
Norris *et al.* 1988	Batch shake flasks, microbial protein assays	*A. ferrooxidans*	<75	7	-
		L. ferrooxidans		87	
Natarajan *et al.* 1992	Batch shake flask, microbial protein assays	*A. ferrooxidans*	63 - 75	60.4	-
			600 -1000	40	
			4000-4750	25	
Ohmura *et al.* 1993	Batch shake flasks, direct cell counts	*A. ferrooxidans*	61.7- 64.6	24	-
Sampson *et al.* 2000	Batch test tube OD readings	*A. ferrooxidans*	32 – 53	25.9	-
		A. ferrooxidans (MA)		28.8	-
Rodriguez *et al.* 2003	Batch shake flasks, direct cell counts	Mixed culture (*Acidithiobacillus, Leptospirillum sp*)	2 - 44	100	Instantaneous (1 minute)
Harneit *et al.* 2005	Batch shake flasks, direct cell counts	*A. ferrooxidans*	50 – 100	85	30
This study	Packed bed reactor, direct cell counts	*A. ferrooxidans* (OF)	(n.a)	52 ± 4.3	120
		A. ferrooxidans (MA)		74 ± 3.8	120
		L. ferriphilum (OF)		55 ± 1.0	120
		L. ferriphilum (MA		79 ± 2.6	120

Mesophilic microorganisms adapted to sulfide (pyrite) mineral concentrate exhibited enhanced attachment efficiencies. The attachment efficiencies were observed to increase from 52 ± 4.3 for ferrous grown cells to 74 ± 3.8 % for pyrite grown cells for *A. ferrooxidans*, and 55 ± 1.0 to 79 ± 2.6 % for *L. ferriphilum* (Table 5.6). The observation that growth history affects subsequent attachment behaviour and more specifically that growth on sulfide mineral concentrate results in enhanced attachment efficiency is consistent with findings of Sampson *et al.* (2000), where attachment of ferrous grown *A. ferrooxidans* to pyrite increased from 25.9 to 28.8 % when cultured on sulfide mineral (chalcopyrite). Although the level of attachment achieved by Sampson *et al.* is lower than that observed in this study, their experimental approach estimated the rate of attachment over a contact period of only 10 minutes.

Sampson *et al.* cultured mineral-adapted microorganisms using chalcopyrite, whereas pyrite mineral concentrate was used in this study.

The results obtained for the attachment of mesophiles, cultured in the absence of (OF), or adapted to (MA) sulfide mineral concentrate, to chalcopyrite and pyrite mineral concentrates are compared in Table 5.8. Trends of attachment observed for the pyrite mineral system were similar to those observed for the chalcopyrite mineral system. For both the pyrite and chalcopyrite mineral systems growth history and culture conditioning was demonstrated to enhance the extent of subsequent attachment. From the results presented in Table 5.8, a trend of higher levels of attachment to pyrite was evident in mineral-adapted cultures with ore-free cultures reaching comparable attachment efficiencies.

Table 5.8: Comparison of attachment efficiencies achieved for mesophilic microorganisms to pyrite and chalcopyrite mineral concentrate systems. The attachment efficiency is presented as a percentage of the inoculum retained in the reactor and per available surface area.

Mineral	**Pyrite**		**Chalcopyrite**	
Attachment efficiency	**(%)**	**(cells m^{-2})**	**(%)**	**(cells m^{-2})**
A. ferrooxidans (OF)	52 ± 4.3	4.5 x10^{10} ± 6 x 10^{9}	52 ± 1.5	2.8 x10^{10} ± 1 x 10^{9}
A. ferrooxidans (MA)	74 ± 3.8	7.5 x10^{10} ± 1 x 10^{10}	63 ± 1.1	5.9 x10^{10} ± 3 x 10^{9}
L. ferriphilum (OF)	55 ± 1.0	2.3 x10^{10} ± 7 x 10^{9}	27 ± 3.4	1.5 x10^{10} ± 3 x 10^{9}
L. ferriphilum (MA)	79 ± 2.6	7.5 x10^{10} ± 3 x 10^{8}	58 ± 2.6	5.6 x10^{10} ± 5 x 10^{9}

Attachment efficiencies of 52 ± 4.3 and 52 ± 1.5 % were reached for ore-free cultured *A. ferrooxidans* and 55 ± 1.0 and 27 ± 3.4 % were achieved for ore-free *L. ferriphilum* to pyrite and chalcopyrite mineral concentrate systems respectively. For mineral-adapted cultures the higher attachment efficiencies of 74 ± 3.8 and 63 ± 1.1 % for *A. ferrooxidans*, and 79 ± 2.6 and 58 ± 2.6 % for *L. ferriphilum* were observed for each respective mineral (Table 5.8). This may lead one to infer that these mesophilic microorganisms attach preferentially to pyrite mineral concentrate over chalcopyrite under these experimental conditions. However, the mineral adaptation, carried out using a pyrite mineral concentrate, may have primed the microorganisms for attachment to pyrite mineral concentrate thus enhancing attachment to pyrite relative to the chalcopyrite mineral concentrate. The effect of growth history on subsequent attachment trends is discussed fully in Section 5.6, subsequent to characterisation of cell surface properties.

5.5 ATTACHMENT OF THERMOPHILES TO SULFIDE MINERAL CONCENTRATE

Experiments to investigate the propensity of *S. metallicus* to attach to chalcopyrite and pyrite mineral concentrate systems, under varying culture conditions, were conducted under ambient conditions typical of heap inoculation. Microorganisms were cultured either in the absence of (OF), or adapted to (MA) solid sulfide (chalcopyrite) mineral particles. The experiments were conducted as described previously, at pH 2.5 and ambient temperature. Data are reported as an average of triplicate results. The standard deviations were large, indicative of poor reproducibility. Cell retention in the column with time, and thereby eluent volume, is presented as a percentage of the inoculum cell number for both chalcopyrite and pyrite mineral systems in Figure 5.5.

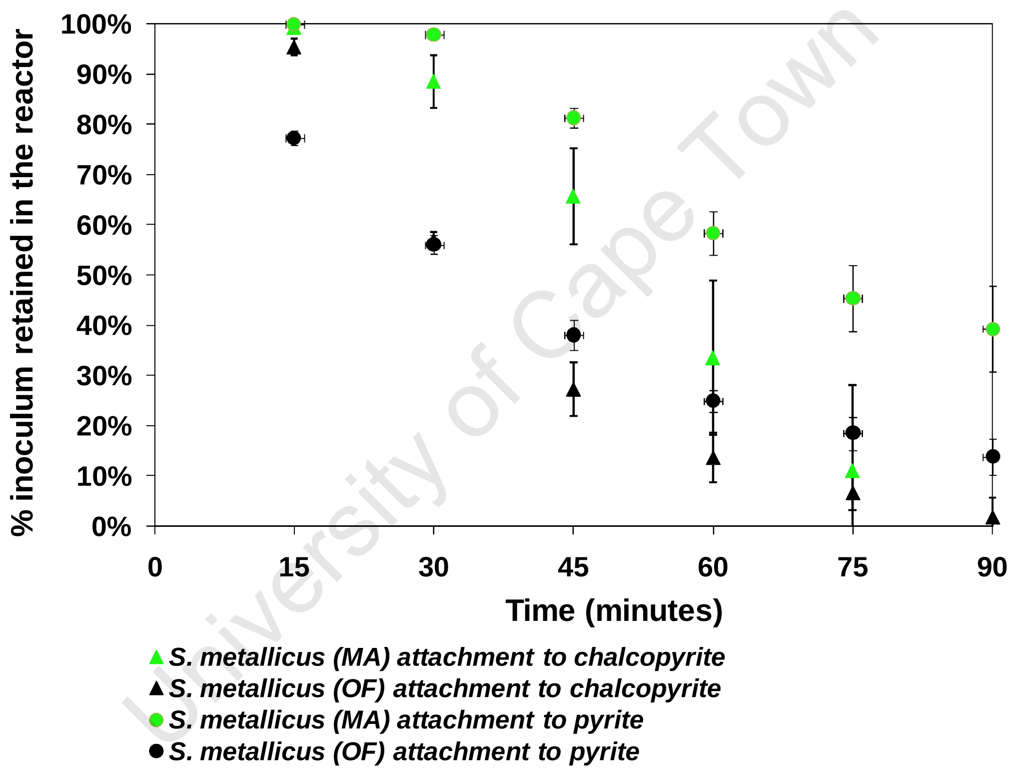

Figure 5.6: Attachment of *S. metallicus* cultured either in the absence of ore (OF) or adapted to sulfide mineral systems (MA), to a chalcopyrite (▲) and pyrite (●) mineral concentrate packed column reactor systems. The duration of the experiment was 135 minutes. However, the results are only represented graphically until wash out was observed.

5.5.1 Attachment of *S. metallicus* to sulfide mineral concentrates: Results

Upon examination of the results of *S. metallicus* (OF) attachment to chalcopyrite, presented in Figure 5.5. There was a steady decrease in the proportion of inoculum that remained in the reactor as the experiment progressed. No plateau of the level of attachment was observed, as washout occurred after 90 minutes. A similar trend is evident for the attachment of *S. metallicus* (OF) to pyrite mineral concentrate, with 14 % of the inoculum being retained at 90 minutes. However, washout occurred at the end of the experiment. The attachment data are summarised in Table 5.9.

Table 5.9: Comparison of attachment efficiencies achieved for *S. metallicus* to chalcopyrite and pyrite mineral concentrate systems. The attachment efficiency is presented as a percentage of the inoculum that remained in the reactor.

Growth history	Time (minutes)	% Inoculum retained	
Ore-free culture		**Chalcopyrite**	**Pyrite**
	30	57 ± 1.6	56 ± 1.9
	45	27 ± 5.3	38 ± 2.9
	60	14 ± 5.0	25 ± 2.1
	90	-	14 ± 3.5
Overall attachment efficiency		0	5 ± 5.0
Mineral-adapted	30	89 ± 5.2	98 ± 0.5
	45	66 ± 9.5	87 ± 1.9
	60	34 ± 15.3	58 ± 4.3
	90	-	31 ± 9.9
Overall attachment efficiency		0	28 ± 9.9

From the results presented in Table 5.9, it is evident that for ore-free cultures the initial retention of cells in the column is similar for chalcopyrite and pyrite mineral systems, with 57 ± 1.6 and 56 ± 1.9 % achieved within the first 30 minutes respectively. In both mineral systems the cell retention within the reactor decreases with time, with the decrease being more substantial for the chalcopyrite than the pyrite system. The retention of inoculum within the reactor decreased to 27 ± 5.3 and 14 ± 5.0 % for the chalcopyrite mineral system and 38 ± 2.9 and 25 ± 2.1 % for the pyrite mineral system after 45 and 60 minutes (one residence time).

Washout was observed in both sulfide mineral systems. Washout occurred more rapidly for the chalcopyrite mineral system, being evident within 90 minutes. After 90 minutes, a cell retention of 14 ± 3.5% was observed in the pyrite mineral system. Retention decreased in the pyrite mineral system with a cell retention of 6 ± 5.7 % observed after two residence times

(120 minutes), and an overall attachment efficiency of 5 ± 5.0 % achieved at 135 minutes. No levelling off or attachment plateau was observed for ore-free cultured *S. metallicus* to either of the sulfide mineral concentrate systems. The higher level of cell retention observed for the pyrite mineral system relative to the chalcopyrite mineral concentrate system may lead one to infer that ore-free cultured *S. metallicus* has a greater affinity for pyrite mineral concentrate, but the association is strictly reversible under attachment conditions used.

The results observed for the attachment of *S. metallicus* cultured in the presence of solid sulfide mineral concentrate are shown on Figure 5.5 and in Table 5.9. High initial cell retention of 89 ± 5.2 % was evident in the chalcopyrite mineral system after 30 minutes. Even higher initial cell retention of 98 ± 0.5 % was observed in the pyrite mineral system after 30 minutes. Thereafter, cell retention decreased rapidly. In the chalcopyrite mineral system, 66 ± 9.5 and 34 ± 15.3 % of the inoculum was retained after 45 and 60 minutes. In the pyrite mineral system, cell retention was higher with 87 ± 1.9 and 58 ± 4.3 % inoculum retained after 45 and 60 minutes. In the chalcopyrite mineral system, a washout of cells from the column was observed within 90 minutes, whereas retention of 31 ± 9.9 % was observed in the pyrite mineral system. Cell retention then levelled off with an overall attachment efficiency of 28 ± 9.9 % for the attachment of mineral-adapted *S. metallicus* to pyrite mineral concentrate. Higher initial cell retention, and higher overall attachment efficiencies observed in the pyrite mineral system relative to the chalcopyrite mineral system suggested that mineral-adapted *S. metallicus* attached preferentially to pyrite mineral concentrate relative to chalcopyrite mineral concentrate.

The trends observed for mineral-adapted *S. metallicus* were similar to that observed for ore-free cultured *S. metallicus*. However, initial cell retention was higher relative to that observed for ore-free culture. No trend of a levelling off of attachment was observed in either the mineral-adapted or ore-free *S. metallicus* – chalcopyrite systems, with wash out observed within 90 and 120 minutes respectively. For the mineral-adapted *S. metallicus* – pyrite system the plateau of the attachment level was approached with overall attachment efficiencies observed to be higher relative to ore-free culture - pyrite system. This illustrates that growth of *S. metallicus* on sulfide minerals enhanced subsequent attachment efficiency to pyrite mineral concentrate.

The solution chemistry was monitored (data given in Appendix C). The pH, redox potential and conductivity of the eluted stream remained within range of the standard solution feed. Attachment efficiencies observed could not be attributed to a change in solution chemistry.

5.5.2 Attachment of *S. metallicus* to sulfide mineral concentrates: Discussion

Overall attachment efficiencies observed for the attachment of both ore-free and mineral-adapted cultures to chalcopyrite and pyrite mineral concentrates were low under these experimental conditions. Washout was observed for ore-free and mineral-adapted *S. metallicus* attachment to the chalcopyrite mineral concentrate system. Washout was also observed for ore-free cultured *S. metallicus* attachment to pyrite mineral concentrate, with mineral-adapted cultures reaching an overall attachment efficiency of 28 ± 9.9 % to pyrite mineral concentrate.

The attachment of thermophilic microorganisms to sulfide minerals has been reported by Sampson *et al.* (2000) and Rodriguez *et al.* (2003). These authors report attachment efficiencies for *Sulfolobus spp.* (grown in the absence of ore) to pyrite mineral concentrate of 64 % and 92 %, using batch agitated systems at 25˚ and 68˚C respectively. Rodriguez *et al.* report an overall attachment efficiency of 96 % for the attachment of ore-free cultured *Sulfolobus spp.* to a chalcopyrite mineral concentrate at 68˚C. The attachment reported by these authors was substantially higher than that observed in this study. Sampson *et al.* suggest that an attachment efficiency of 64 % for the attachment of *Sulfolobus spp.* (ore-free cultured) to a pyrite mineral concentrate may underestimate maximum attachment levels attainable by these microorganisms to pyrite as the experiment was conducted at sub-optimal temperature conditions. The latter holds for this study too. This, and the flow through nature of the system, are expected to decrease attachment efficiency but are also more representative of heap inoculation.

The lower levels of attachment observed for *S. metallicus* to sulfide minerals relative to that of literature reports may be due to differences in experimental approach. Literature reports made use of batch agitated systems, whereas a continuous flow through packed column reactor was used in this study. Thus, lower levels of attachment were expected for ore-free cultured *S. metallicus* in this study relative to literature reports as the forces imposed by continuous fluid flow had to be overcome for attachment to occur. The extent of the discrepancy is quite substantial as washout was observed for most of the experimental runs. It is inferred that this may be due to the contribution of sub-optimal temperature conditions during inoculum preparation (cells were harvested at 4 ˚ C) and throughout the experiment (experiment was conducted at ambient conditions) which may have hampered the activity of the cells and thus impeded extent of the attachment attainable by *S. metallicus* under these experimental conditions. Attachment has also been reported to be strain specific[1] and affected by microbial activity[2] (Sampson *et al.* 2000[1], Zobell 1943[2], van Loosdrecht *et al.* 1987 and 1990[1, 2], and Rijnaartss *et al.* 1993[1, 2]). It is likely that the *S. metallicus* culture may have lost its ability or affinity to attach to solid sulfide minerals due to laboratory culture maintenance conditions.

This may explain the decreased levels of attachment observed. These factors, together with column effects (channelling and the effect of continuous fluid flow) may have contributed to the washout observed.

The results observed for attachment of *S. metallicus* were substantially lower than the attachment observed for ore-free and mineral-adapted mesophilic cultures to the respective sulfide mineral systems. Mesophilic microbial attachment to sulfide minerals exhibited slow initial decrease in the proportion of cells retained in the reactor followed by an attachment plateau. This trend was observed for the mineral-adapted *S. metallicus* attachment to pyrite mineral concentrate. In the mesophilic systems enhanced attachment efficiencies were observed for mineral-adapted cultures over ore-free cultures. For *S. metallicus*, this trend was only observed under equilibrium conditions for the pyrite mineral system. However, the high levels of initial cell retention observed for the mineral-adapted *S. metallicus* – chalcopyrite system relative to the ore-free cultures may be indicative of enhanced affinity for this mineral. These findings may be due to the microbial strain, microbial activity, and column and temperature effects previously discussed.

Table 5.10: Attachment efficiencies observed for *A. ferrooxidans*, *L. ferriphilum* and *S. metallicus* to chalcopyrite and pyrite mineral concentrate in packed column reactor systems as a function of culture conditions.

Microbial growth history	Attachment efficiency (%) as a function of mineral	
Ore-free cultured	**Chalcopyrite**	**Pyrite**
A. ferrooxidans	52 ± 1.5	52 ± 4.3
L. ferriphilum	27 ± 5.3	55 ± 1.0
S. metallicus	0	5 ± 5.0
Mineral-adapted		
A. ferrooxidans	63 ± 1.1	74 ± 3.8
L. ferriphilum	58 ± 2.6	79 ± 2.6
S. metallicus	0	28 ± 9.9

The standard deviations reported for *S. metallicus* attachment in these studies were, at times, unacceptably high, indicative of large level variation across the three columns used. Difficulty experienced in enumerating these microorganisms using direct microscopic counting is expected to have aggravated the variability.

Pyrite mineral-adapted mesophilic microorganisms exhibited enhanced attachment efficiencies to sulfide minerals, with the most significant improvement in attachment observed to pyrite mineral concentrate. Thus it was inferred that attachment could be primed using culture conditioning with particular minerals. It was expected that following mineral adaptation of *S. metallicus* using chalcopyrite mineral concentrate, greater attachment of mineral-adapted *S. metallicus* to chalcopyrite would be observed. However, highest attachment efficiencies were observed to pyrite mineral concentrate. These findings are consistent with Sampson *et al.* (2000) who reported enhanced levels of attachment to pyrite over chalcopyrite for chalcopyrite cultured *Sulfolobus spp.* (TH1 strain). This was substantiated using XLDVO theory and cell surface hydrophobicity measurements with calculation of the free energy of adhesion of ferrous or chalcopyrite grown *Sulfolobus spp.*

Chalcopyrite grown *Sulfolobus spp.* exhibited increased cell surface hydrophobicity (contact angle measurements) relative to ferrous grown cultures (28.6 ± 5.7 and 48.4 ± 9.5 for ferrous and chalcopyrite grown cultures (TH1 strain) respectively). Chalcopyrite mineral has a greater surface hydrophobicity than pyrite mineral (Ohmura *et al.,* 1993). Due to the hydrophobicity of both chalcopyrite and the microbial surface , the surfaces were expected to would associate in an aqueous environment enhancing attachment to chalcopyrite over pyrit, contrary to observation. Yet, the opposite was observed. The observed trend may have been due the contribution of other surface interactions, such as surface charge. The effect of growth media on cell surface charge is presented in Section 5.6.

5.6 ZETA-POTENTIAL OF MICROBIAL SPECIES GROWN UNDER MINERAL-ADAPTED CULTURE CONDITIONS

5.6.1 Results

The zeta potential of the microorganisms was measured in a cell culture with a concentration of 2 x 10^8 cells ml^{-1} suspended in 9 K medium at pH 2.5. The pH 2.5 was selected since attachment studies were conducted at this pH. Results reported are an average of at least four measurements. The standard deviations of these results were small demonstrating good reproducibility. The surface charge of ore-free and mineral-adapted microbial species observed in this study, together with that reported in literature, is presented in Table 5.11. Where electrokinetic potentials were not available from literature, the isoelectric point (IEP) and corresponding pH are given. From analysis of these results, all mineral-adapted microorganisms exhibited a net positive cell surface charge in 9 K media at pH 2.5. Microorganisms cultured in the absence of sulfide mineral concentrate exhibited a net negative cell surface charge, with the exception of *L. ferriphilum*. These findings illustrate that growth history influences cell surface properties.

Table 5.11: Summary of the electrokinetic potential measured at pH 2.5, of cells cultured in the absence of sulfide mineral concentrates (OF) or adapted to sulfide mineral concentrate (MA) contrasted with literature reports.

Author	Microorganism	OF ζ (mV)	MA ζ (mV)
Africa *et al.*	*A. ferrooxidans*	-1.76 ± 1.02	-
	L. ferriphilum	2.78 ± 0.45	3.48 ± 0.32
	S. metallicus	-0.48 ± 0.16	2.29 ± 0.85
Devasia *et al.* 1993	*A. ferrooxidans*	IEP pH 2.0	IEP pH 3.8
Ohmura *et al.* 1993	*A. ferrooxidans*	- 4.6 at pH 2.0	-
Blake *et al.* 1994	*A. ferrooxidans*	- 5.0 + 1.5 at pH 2.0	-
Sharma *et al.* 2003	*A. ferrooxidans*	IEP ± pH 2.0	IEP pH 3.5

The negative cell surface charge, measured at pH 2.5, observed for *A. ferrooxidans* cultured in the absence of sulfide mineral concentrate is in accordance with reports in literature (Devasia *et al.* 1993, Ohmura *et al.* 1993, Blake *et al.* 1994 and Sharma *et al.* 2003). The observation of a net positive cell surface charge exhibited by mineral-adapted microbial species is in accordance with literature reports of Devasia *et al.* (1993) and Sharma *et al.* (2003) for *A. ferrooxidans* cultured in the presence of sulfide minerals. Literature data are limited to *A. ferrooxidans*.

5.6.2 Discussion

The adsorption trends observed are discussed with respect to forces responsible for the initial attachment stage, these being transient, reversible electrostatic and hydrophobic interactions between the mineral and microbial surfaces (Zobell 1943, van Loosdrecht *et al*. 1987, van Loosdrecht *et al.* 1990, Rijnaarts *et al.* 1993, Porro *et al.* 1997*,* Blake *et al.* 1994, Nagaoka *et al.* 1994, Sampson *et al.* 2000, Sharma *et al.* 2003). These depend on mineral and microbial surface properties (surface charge and hydrophobicity). From the zeta-potential measurements of microorganisms grown in the absence of, or adapted to, sulfide mineral concentrate, changes in the cell surface charge are evident. Mineral-adapted cells exhibited a positive cell surface charge whereas ore-free cultured microorganisms exhibited a negative cell surface charge.

The results of this study demonstrated that culture conditioning affected cell surface charge. The effect of culture conditioning on the alteration of cell surface properties has been reported previously (Porro *et al.* 1993, Rijnaarts *et al.* 1993, Blake *et al.* 1994, Gerhke *et al.* 1998, Kinzler *et al.* 2003, Sharma *et al.* 2003 and Harneit *et al.* 2005). It has been demonstrated that cells grown on solid media exhibit enhanced production of EPS (Vandevivere *et al.* 1993, Gerhke *et al.* 1998, Kinzler *et al.* 2003, Harneit *et al.* 2005). Assuming EPS production was enhanced, the positive cell surface charge exhibited by mineral-adapted cultures may be due to the complexation of ferric ions within the EPS layer (Gehrke et al. 1998, Kinzler *et al.* 2003, Harneit *et al.* 2005).

Chalcopyrite is neutral to positively charged and pyrite is negatively charged, with the surface of chalcopyrite being more hydrophobic relative to pyrite under the conditions of this experiment (Devasia *et al.* 1993, Ohmura *et al.* 1993, Nagaoka *et al.* 1999) discussed in Section 2.4.4. *A. ferrooxidans* cultured in the absence of solid sulfide mineral ore exhibit a negative surface charge (Ohmura *et al.* 1993, Nagoaka *et al.* 1994, Sharma *et al.* 2003) and is also relatively hydrophobic (Ohmura et al. 1993, Devasia *et al.* 1993, Nagaoka *et al.* 1994, Blake *et al.* 1994, Sharma *et al.* 2003) discussed in Section 2.4.3. In an aqueous environment the contribution of hydrophobic interactions in initial adhesion is believed to be more substantial (van Loosdrecht *et al.* 1987, Rijnaarts *et al.* 1993, Blake *et al.* 1994, and Sharma *et al.* 2003) relative to electrostatic interactions discussed in Section 2.4.3 and 2.4.4. Thus, despite the predicted electrostatic repulsion between the pyrite and ore-free cultured microbial surfaces, the surfaces would tend to associate since both are hydrophobic.

For the chalcopyrite and pyrite mineral systems, enhanced attachment efficiencies were observed for cultures grown in the presence of sulfide mineral concentrate relative to ore-free cultures as presented in Table 5.10, for *A. ferrooxidans*, *L. ferriphilum* and *S. metallicus.* The alteration in the cell surface properties for cultures grown in the presence of sulfide mineral concentrate supports the enhanced attachment efficiencies observed. A net positive cell surface charge exhibited by mineral-adapted cultures increases the electrostatic attraction between microbial and mineral surfaces under these experimental conditions. Growth on sulfide minerals has been demonstrated to enhance cell surface hydrophobicity (Porro *et al.* 1993, Blake *et al.* 1994, Sampson *et al.* 2000, Sharma *et al.* 2003). Thus; the extent of the attractive hydrophobic forces between microbial and mineral surfaces would also have been enhanced for both mineral systems. Greater attachment efficiencies for mineral-adapted cultures to sulfide mineral concentrates were therefore expected. The enhancement of the mineral-adapted mesophilic microbial attachment is more substantial for the pyrite mineral system relative to chalcopyrite mineral system. This is likely due to the fact that the extent of the attractive electrostatic forces between microbial and pyrite mineral surfaces would be greater than that experienced between microbial and chalcopyrite mineral surfaces under these experimental conditions. Also, mineral adaptation for mesophilic microorganisms was carried out using a pyrite mineral concentrate, which may have primed microorganisms for attachment to this mineral system explaining the enhanced levels of attachment to the pyrite mineral system relative to chalcopyrite.

The findings of this study for mesophilic microorganisms are consistent with Gehrke *et al.* (1998), with observations for thermophiles consistent with reports by Sampson *et al.* (2000). Selective attachment using quartz as a control mineral will be discussed fully in Section 5.7.3.

5.7 INVESTIGATION OF THE PREVALENCE OF SELECTIVE ATTACHMENT OF MESOPHILES TO SULFIDE MINERALS RELATIVE TO THE QUARTZ CONTROL

Experiments to investigate the prevalence of selective attachment of *A. ferrooxidans* and *L. ferriphilum* to sulfide minerals were conducted using chalcopyrite and pyrite mineral concentrates, a low-grade chalcopyrite containing mineral ore, and quartz, which served as a control mineral. The effect of growth history on attachment was considered.

5.7.1 Attachment of *A. ferrooxidans* and *L. ferriphilum* to quartz: Results

Cell retention in the column with time and elution flow is given as a percentage of the inoculum cell concentration in Figure 5.6 and summarised in Table 5.12. Upon examination of these results similar attachment trends were evident for ore-free cultured *A. ferrooxidans* and *L. ferriphilum*. For both cultures the rate and extent of the cell retention within the column was inversely proportional to that of the cells washing out of the column. For *A. ferrooxidans,* there was high level of initial cell retention with 98 ± 0.6 % retained after the first 30 minutes. The retention then decreased slowly to 85 ± 4.4 % at 45 minutes, equivalent to the passage of 45ml eluent. Subsequently, a rapid decline in cell retention was observed with 58 ± 5.8 % of the inoculum retained after the first residence time. An attachment plateau was attained toward the second residence time. Cell retention of 36 ± 0.5 % was observed after the second residence time. An overall attachment efficiency of 35 ± 0.8 % was attained at 135 minutes for *A. ferrooxidans* attachment to quartz.

Attachment of ore-free cultured *L. ferriphilum* to the quartz mineral system followed a similar trend. However, the extent of the cell retention was higher for *L. ferriphilum* relative to *A. ferrooxidans*, with 97 ± 0.5 % inoculum retention observed after 30 minutes and 92 ± 0.6 % after 45 minutes. Cell retention was higher for *L. ferriphilum* relative to *A. ferrooxidans* with 82 ± 3.1 % and 54 ± 4.2 % inoculum retained after one and two residence times respectively. An overall attachment of 51 ± 4.5 % was observed for *L. ferriphilum,* compared to 36 ± 0.5 % for *A. ferrooxidans.* While cell retention appeared to be levelling off, an attachment plateau was not attained within two residence times and cell retention continued to decrease. Thus the final attachment efficiency observed for *L. ferriphilum* may be an overestimate of the maximum efficiency attainable for this microorganism under these experimental conditions.

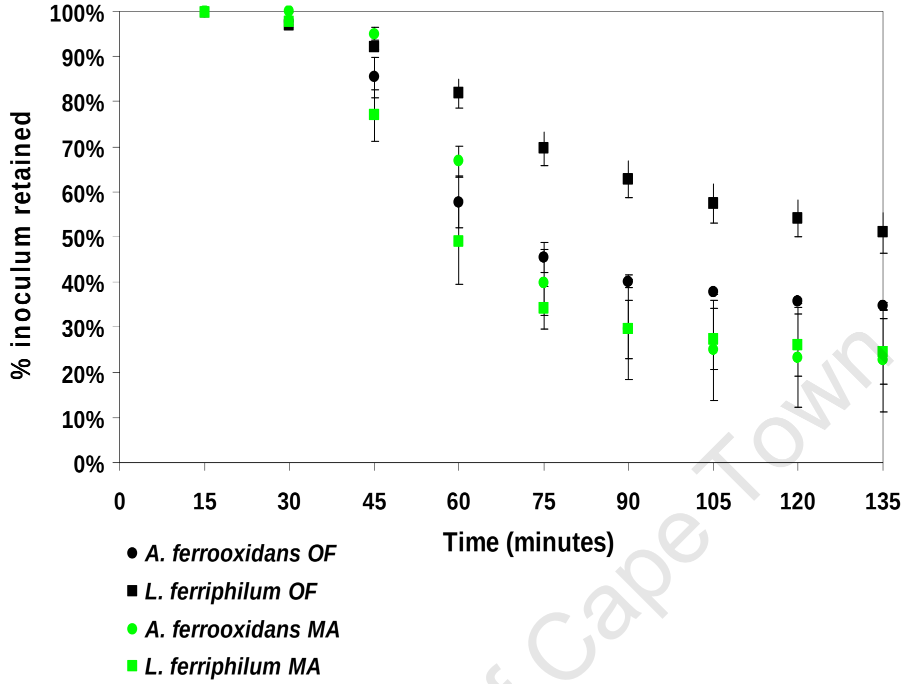

Figure 5.7: Attachment of *L. ferriphilum* and *A. ferrooxidans* cultured either in the absence of ore (OF) or adapted to sulfide mineral systems (MA), to quartz in the packed column reactor system. The change in cells retention in the column with time and eluent volume is represented as a percentage of the inoculum remaining in the column. Samples were taken in 15 ml aliquots with the reactor operated at 1ml min^{-1}.

For the attachment of mineral-adapted *A. ferrooxidans* and *L. ferriphilum* to quartz, the same trend of retention observed for ore-free cultures was evident for mineral-adapted cultures upon analysis of the results presented in Table 5.12 and Figure 5.6. The initial level of cell retention was high with 100 ± 0.1 and 98 ± 0.8 % observed after 30 minutes, and 95 ± 1.5 and 77 ± 5.7 % observed after 45 minutes for *A. ferrooxidans* and *L. ferriphilum* respectively. A rapid decrease in cell retention followed, with cell retention levelling off after two residence times. Cell retention was 67 ± 3.4 and 23 ± 11.1 %, and 49 ± 9.3 and 26 ± 6.9 % after one and two residence times for *A. ferrooxidans* and *L. ferriphilum* respectively. Overall attachment efficiencies were lower for mineral-adapted cultures than ore-free cultures with 23 ±11.3 and 25 ± 7.2 % observed for *A. ferrooxidans* and *L. ferriphilum* respectively, confirming that growth history affected subsequent attachment behaviour.

Table 5.12: The change in cell retention within a quartz packed column reactor as the experiment progressed is presented for *A. ferrooxidans* and *L. ferriphilum.* The overall attachment efficiency is presented as the percentage inoculum retained in the reactor after 135 minute experiment.

Growth history	Time (minutes)	*A. ferrooxidans*	*L. ferriphilum*
Ore-free culture		% Inoculum retained	
	30	98 ± 0.6	97 ± 0.5
	45	85 ± 4.4	92 ± 0.6
	60	58 ± 5.8	82 ± 3.1
	120	36 ± 0.5	54 ± 4.2
Overall attachment efficiency		35 ± 0.8	51 ± 4.5
Mineral-adapted		% Inoculum retained	
	30	100 ± 0.1	98 ± 0.8
	45	95 ± 1.5	77 ± 5.7
	60	67 ± 3.4	49 ± 9.3
	120	23 ± 11.1	26 ± 6.9
Overall attachment efficiency		23 ± 11.1	25 ± 7.2

The pH, redox potential and conductivity of the standard (9 K) feed solution used were pH 2.45, 371 mV and 5.57 mS. The average pH, redox potential and conductivity of solution passing through the three columns ranged from pH 2.45 to 2.49, 404 mV to 533 mV and 5.42 to 5.60 mS. The pH, redox potential and conductivity of the exiting solution was within range of the standard feed solution, thus attachment observed for mesophilic-quartz mineral systems was not due to a change in solution chemistry. (Data appended in Appendix C).

5.7.2 Attachment of *A. ferrooxidans* and *L. ferriphilum* to low-grade chalcopyrite containing mineral ore: Results

The retention of the mesophiles in columns packed with low-grade ore coated beads with time is presented in Figure 5.8 and summarised in Table 5.13. A similar trend was evident for ore-free cultured *A. ferrooxidans* and *L. ferriphilum*, with differing, the extents of the cell retention and attachment observed between microorganisms. High initial cell retention is evident with 99 ± 0.3 and 98 ± 0.6 % inoculum retained after 30 minutes for *A. ferrooxidans* and *L. ferriphilum* respectively. Subsequently, retention decreased progressively with time. The decrease was greater for *A. ferrooxidans* than *L. ferriphilum.* Cell retention after one and two residence times was determined as 51 ± 12.6 and 10 ± 2.2 % for *A. ferrooxidans*, and 78 ± 1.3 and 35 ± 1.6 % for *L. ferriphilum.* Final attachment efficiencies observed were 7 ± 4.7 and 26 ± 1.5 % for *A. ferrooxidans* and *L. ferriphilum* respectively. An attachment plateau was not observed to be approached within the time span of the experiment. This leads one to infer that the attachment efficiencies reported overestimate the maximum level of attachment to chalcopyrite whole ore attainable under these experimental conditions.

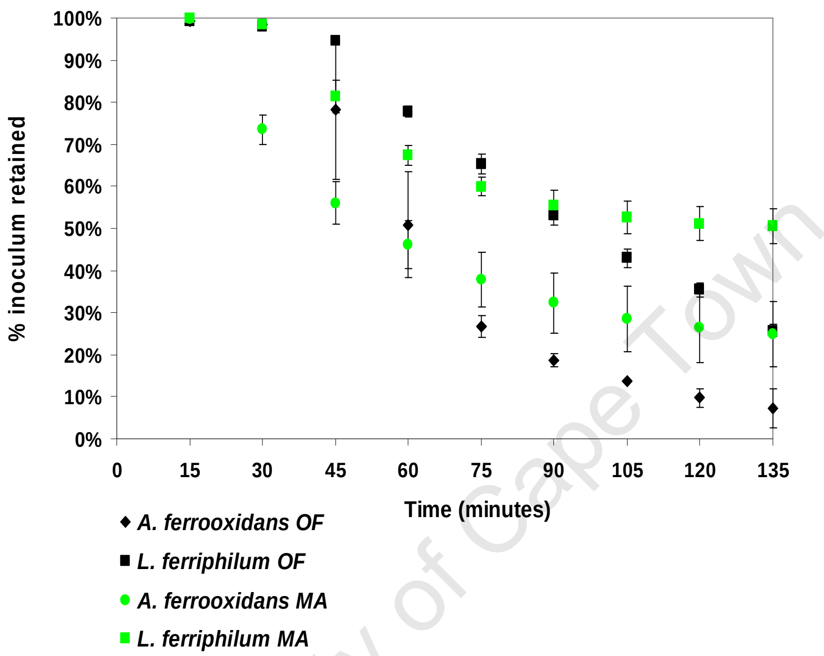

Figure 5.8: Attachment of *L. ferriphilum* and *A. ferrooxidans* cultured either in the absence of ore (OF) or adapted to sulfide mineral systems (MA), to low-grade-chalcopyrite mineral ore in the packed column reactor system.

Similar trends were evident for mineral-adapted *A. ferrooxidans* and *L. ferriphilum*, with differences in the extent of the cell retention and subsequent attachment efficiency observed between microorganisms (Figure 5.7, Table 5.13). High initial cell retention is evident for *L. ferriphilum* with 98 ± 1.3 % inoculum retained after 30 minutes. A lower initial cell retention of 73 ± 3.5 % was observed for *A. ferrooxidans.* Subsequently the level of cell retention decreased at similar rates for both microorganisms. The cell retention decreased through 56 ± 5.0 to 46 ± 5.7 % for *A. ferrooxidans*, and through 81 ± 3.9 to 67 ± 2.4 % for *L. ferriphilum* after 45 and 60 minutes respectively. An attachment plateau was approached after two residence times with 26 ± 8.2 and 51 ± 4.0 % inoculum retained for *A. ferrooxidans* and *L. ferriphilum* respectively. The overall attachment efficiency for *A. ferrooxidans* of 25 ± 7.9 % was observed to be half that for *L. ferriphilum* of 50 ± 4.1 %.

Distinct trends of attachment were observed for attachment of mineral-adapted cultures to low-grade chalcopyrite ore relative for that observed for ore-free cultures. For mineral-adapted mesophiles, cell retention levelled off after two residence times. This was not the case for ore-free cultures and cell retention continued to decrease. Higher attachment efficiencies were observed for mineral-adapted cultures relative to ore-free cultures. This may lead one to infer that growth history affects subsequent attachment behaviour.

Table 5.13: Retention of *A. ferrooxidans* and *L. ferriphilum.* within the packed column reactor system containing low-grade chalcopyrite mineral ore.

Growth history	**Time (minutes)**	% Inoculum retained	
Ore-free culture		***A. ferrooxidans***	***L. ferriphilum***
	30	99 ± 0.3	98 ± 0.6
	45	78 ± 16.6	95 ± 1.0
	60	51 ± 12.6	78 ± 1.3
	120	10 ± 2.2	35 ± 1.6
Overall attachment efficiency		7 ± 4.7	26 ± 1.5
Mineral-adapted	30	73 ± 3.5	98 ± 1.3
	45	56 ± 5.0	81 ± 3.9
	60	46 ± 5.7	67 ± 2.4
	120	26 ± 8.2	51 ± 4.0
Overall attachment efficiency		25 ± 7.9	50 ± 4.1

The pH, redox potential and conductivity of the standard (9 K) feed solution used were pH 2.46, 476 mV and 5.57 mS for the *L. ferriphilum*-low-grade chalcopyrite mineral system. The average pH, redox potential and conductivity of solution passing through the three columns ranged from pH 2.48 to 2.55, 420 mV to 518 mV and 5.37 to 5.49 mS. The pH, redox potential and conductivity of the exiting solution was within range of the standard feed solution, thus attachment observed for mesophilic-low-grade chalcopyrite mineral systems was not due to a change in solution chemistry (Appendix C).

5.7.3 Attachment of *A. ferrooxidans* and *L. ferriphilum* to quartz and low-grade ore: Discussion

The attachment efficiencies of mesophilic microorganisms to quartz reported in literature contrasted with the results of this study in Table 5.14. The attachment efficiencies of ore-free cultured mesophiles were within the range reported by Harneit *et al.* (2005) who reported efficiencies of 18 % after 60 minutes with the establishment of attachment equilibrium of 50 % after 480 minutes using batch agitated systems. Ohmura *et al.* (1993) reported an attachment efficiency of 5 % after a 6 minute contact period, but did not provide equilibrium data to allow comparison with current data. To date no studies have been conducted to investigate the attachment of *A. ferrooxidans* and *L. ferriphilum* to low-grade chalcopyrite containing mineral ore.

Table 5.14: Attachment efficiencies of mesophilic microorganisms to quartz reported in literature contrasted with the results of this study.

Author	**Method**	**Microorganism**	**Particle size** (µm)	**Attachment efficiency** (%)	**Time to equilibrium** (mins)
Ohmura *et al.* 1993	Batch test tube, direct cell counts	*A. ferrooxidans*	61.7-64.6	4.7	-
Harneit *et al.* 2005	Batch shake flasks, direct cell counts	*A. ferrooxidans*	50 – 100	18 (after 60 mins)	
				50	480
Africa *et al.*	Packed bed reactor, direct cell counts	*A. ferrooxidans* (OF)	(n.a)	35 ± 0.8	120
		A. ferrooxidans (MA)		23 ± 11.3	120
		L. ferriphilum (OF)	(n.a.)	51 ± 4.5	120
		L. ferriphilum (MA)		25 ± 7.2	120

The greater attachment observed for ore-free cultures to quartz relative to mineral-adapted cultures can be explained through electrostatic and hydrophobic interactions between the microbial and mineral surfaces. According to literature, quartz has a negative surface charge (Escobar *et al.* 1997 and Sharma *et al.* 2003) and is hydrophilic (Ohmura *et al.* 1993) under the conditions of this experiment. Ore-free cultures exhibited a negative cell surface charge, with mineral-adapted cultures exhibiting positive surface charge. Based on charge, mineral-adapted cultures would be expected to attach preferentially to quartz. However, in an aqueous environment the contribution of hydrophobic interactions in initial adhesion are postulated to dominate over electrostatic interactions in initial adhesion mechanisms (van Loosdrecht *et al.* 1987, Rijnaarts *et al.* 1993, Blake *et al.* 1994, Sharma *et al.* 2003). Mineral-adapted cultures have been demonstrated to exhibit increased hydrophobicity relative to ore-free cultures (Porro *et al.* 1993, Blake *et al.* 1994, Sampson *et al.* 2000, Sharma *et al.* 2003).Thus based on hydrophobic interactions between mineral and microbial surfaces, mineral-adapted cells are expected to associate less with the hydrophilic quartz surface under the conditions of this experiment, than ore-free cultures as observed.

Low attachment efficiencies to low-grade ore observed for ore-free cultures were expected as the low-grade chalcopyrite ore consisted predominantly of gangue minerals, with the predominant gangue minerals being quartz (44.8 wt %) and muscovite (28.6 wt %). The electrostatic and hydrophobic interactions between microbial and mineral surfaces would be comparable to that observed for attachment to quartz, thus the attachment efficiencies are similarly mediocre.

5.7.4 The prevalence of selectivity in microbial attachment to sulfide minerals

The efficiencies observed for attachment of *A. ferrooxidans* and *L. ferriphilum* to chalcopyrite and pyrite mineral concentrates, low-grade chalcopyrite mineral containing ore and quartz are presented in Table 5.15, with greater attachment efficiencies are evident for the sulfide mineral systems relative to the control mineral quartz.

Table 5.15: Attachment efficiency of *A. ferrooxidans* and *L. ferriphilum* grown under varying culture conditions, to chalcopyrite and pyrite mineral concentrate, low-grade chalcopyrite mineral containing ore and quartz in a packed column reactor.

Microbial growth history	**Mineral system investigated**			
	Chalcopyrite (%)	**Pyrite (%)**	**Low-grade chalcopyrite (%)**	**Quartz (%)**
Ore-free cultured				
A. ferrooxidans	52 ± 1.5	52 ± 4.3	7 ± 4.7	35 ± 0.8
L. ferriphilum	27 ± 3.4	55 ± 1.0	26 ± 1.5	51 ± 4.5
Mineral-adapted				
A. ferrooxidans	63 ± 1.1	74 ± 3.8	25 ± 7.9	23 ± 11.3
L. ferriphilum	58 ± 2.6	79 ± 2.6	50 ± 4.1	25 ± 7.2

In order to elucidate trends of preferential attachment of mesophilic microbial species to a particular mineral, a comparison of the data sets obtained for the attachment of *A. ferrooxidans* and *L. ferriphilum* to the various mineral is represented in Figure 5.9. This comparison was accomplished by normalising the data against the control data set, namely attachment to quartz. Normalisation of the data was achieved by dividing the results obtained in each attachment experiment, at the equivalent time, by the corresponding result obtained in the control experiment as follows:

$$Normalised \quad attachment \quad efficiency = \frac{attachment \quad efficiency \quad \exp eriment_{\ time\ x}}{attachment \quad efficiency \quad control_{\ time\ x}}$$

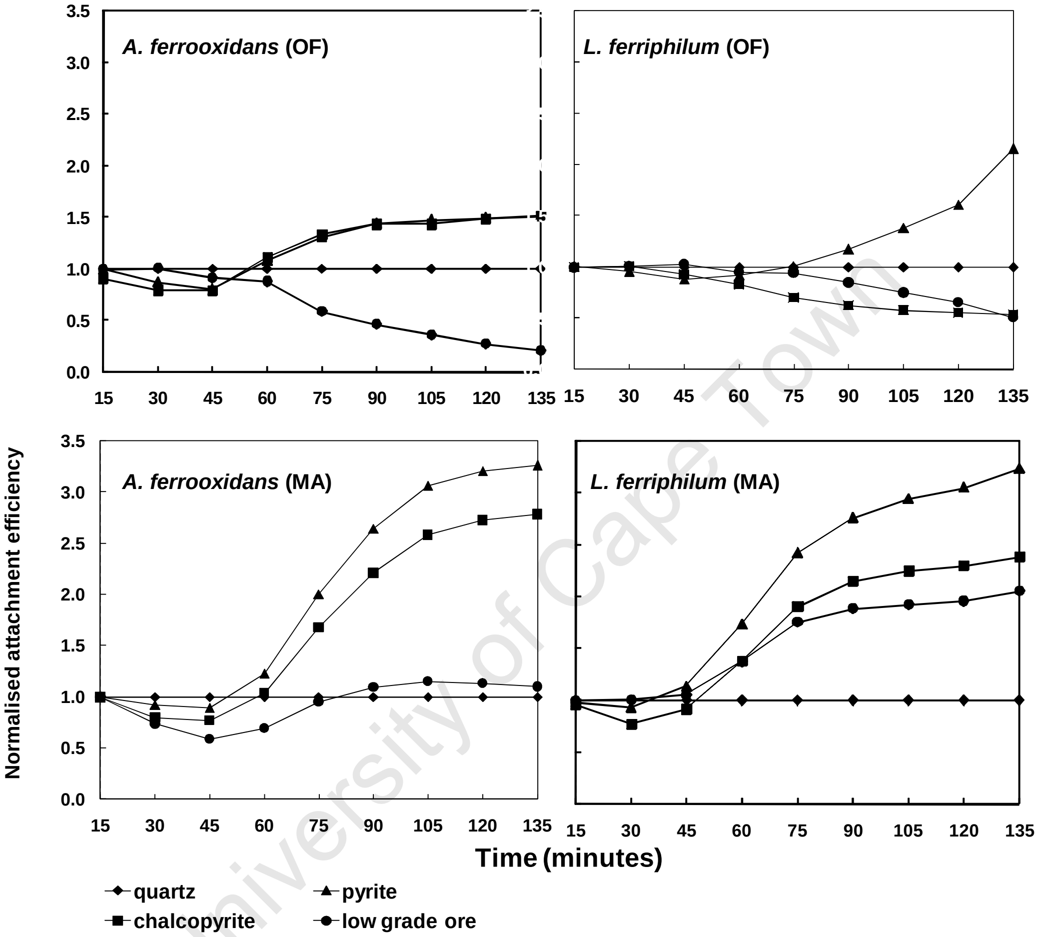

Figure 5.9: Normalised attachment efficiency of *A. ferrooxidans* (left) and *L. ferriphilum* (right) cultured in the absence of sulfide mineral ore (top) and adapted to sulfide mineral ore (bottom).

Upon examination of the normalised data in Figure 5.9, it was evident that, with the exception of *L. ferriphilum*, the attachment of ore-free cultured mesophilic microorganisms was selective for pyrite, followed by chalcopyrite relative to the control mineral quartz. Attachment to low-grade chalcopyrite mineral ore was consistently lower than that observed for the quartz control mineral system at each respective time point. For the attachment of mineral-adapted mesophilic microorganisms to sulfide mineral systems, a similar trend of selective attachment to pyrite followed by chalcopyrite, low-grade ore and then quartz is evident, with the extent of the attachment observed being greater. For *A. ferrooxidans,* attachment appeared to be selective to pyrite, followed by chalcopyrite relative to the control mineral quartz. For *A.*

ferrooxidans, attachment to low-grade chalcopyrite mineral ore was comparable to that observed for the quartz control mineral system.

The trends of selective attachment are corroborated by statistical analysis of the variance of the attachment efficiencies observed for *A. ferrooxidans* and *L. ferriphilum* across all mineral systems using the ANOVA statistical function in Excel (see Appendix D for full statistical analysis). Since the F value of 6.124 was greater that the $F_{critical}$ value of 1.839, with a 95 % confidence and a p value of 0.05, the null hypothesis is rejected (that there is no significant difference in the extent of attachment attained between the various mineral systems) in favour of the alternate. In conclusion there is a statistically significant difference between the extents of attachment observed between various mineral systems.

Selective attachment to pyrite mineral over chalcopyrite and quartz has been reported by Solari *et al.* (1992), Ohmura *et al.* (1993), Nagaoka *et al.* (1999), Gonzalez *et al.* (1999), Sampson *et al.* (2000), Rodriguez *et al.* (2003), Harneit *et al.* (2005) following batch tests in well mixed systems without the replacement of the liquid phase. The results of this study are consistent with previous findings.

5.8 CONCLUSIONS

Microbial adhesion to solid surfaces is a four stage process: initial attachment, followed by firm attachment, colonisation and finally biofilm formation. In this study, initial adhesion interactions were the focus. This involves transport of the microorganism to the mineral surface followed by transient electrostatic and hydrophobic interactions between the surfaces before the onset of firm attachment.

The packed column approach provided an effective means of investigating attachment of microorganisms in a heap-like environment. To date, the most commonly used approach for investigating microbial attachment to minerals has been through the use of batch agitated systems. Hydrodynamic conditions experienced in batch agitated systems differ to those encountered in a heap. Shake flask systems present a well-mixed environment which facilitates contacting of the microbe and mineral. However, shear and attrition forces for microbial detachment are also greater. Hence, the results observed may not necessarily provide a reliable representation of attachment in a heap environment. In this study, the impact of fluid flow has been shown to influence initial attachment behaviour and subsequent attachment efficiencies. The column attachment approach simulated heap-like fluid dynamics, an important factor overlooked by investigation of attachment to date. It has been demonstrated to be an effective means of investigating attachment under heap-like conditions.

For the investigation of the attachment of mesophilic microorganisms to all mineral systems, attachment generally levelled off after 2 residence times (120 minutes) under these experimental conditions. Inoculum concentration is of limited importance in governing the extent of initial adhesion interactions and thus subsequent attachment efficiencies under these experimental conditions as the saturation cell number was never exceeded and the maximum coverage provided by the highest inoculum cell concentration used was only 7 % of the total surface area available for attachment. In all cases, it was found that the attachment results were not due to a change in solution chemistry.

Attachment to sulfide mineral concentrates was demonstrated *A. ferrooxidans* and *L. ferriphilum* cultured in an ore-free environment, with attachment efficiencies of 52 ± 1.5 and 52 ± 4.3 % observed to chalcopyrite and pyrite concentrates by *A. ferrooxidans,* and 27 ± 3.4 and 55 ± 1.0 % observed to chalcopyrite and pyrite concentrates by *L. ferriphilum*. The microorganisms exhibited similar attachment trends, and affinities for attachment to the sulfide mineral concentrates. This was corroborated by statistical analysis of the variances across results observed.

Growth conditioning of the cultures was demonstrated to affect cell surface charge. The cultures adapted to pyrite mineral concentrate exhibited a positive cell surface charge while microorganisms cultured in the absence of ore exhibited a negative cell surface charge. The growth conditioning of cultures using pyrite mineral adaptation and subsequent change in cell surface charge was correlated to an enhancement in the attachment efficiencies observed for all mineral-adapted mesophilic cultures to sulfide mineral concentrates relative to ore-free cultures. For mineral-adapted *A. ferrooxidans,* attachment efficiencies of 63 ± 1.1 and 74 ± 3.8 % were observed for the attachment to chalcopyrite and pyrite while 58 ± 2.6 and 79 ± 2.6 % were observed for each respective mineral system by *L. ferriphilum*. Surface properties, such as surface charge and hydrophobicity of minerals and microorganisms, have been reported to play a significant role in the initial adhesion mechanism (Zobell 1943, van Loosdrecht *et al*. 1987, van Loosdrecht *et al.* 1990, Rijnaarts *et al.* 1993, Porro *et al.* 1997, Blake *et al.* 1994, Nagaoka *et al.* 1994, Sampson *et al.* 2000, Sharma *et al.* 2003). A net positive cell surface charge and increased cell surface hydrophobicity following culturing on sulfide mineral concentrates was hypothesised to increase the extent of the attractive electrostatic interactions between microbial and mineral surfaces resulting in the enhanced attachment efficiencies observed for mineral-adapted cultures.

The highest overall attachment was observed for mineral-adapted mesophilic cultures to pyrite mineral concentrate, explicable due to mineral adaptation being conducted through the use of a pyrite mineral concentrate. Growth conditioning of cells on particular sulfide minerals may prime cells for attachment by enhancing initial attachment interactions and thus overall attachment efficiencies to sulfide minerals. It may be possible to enhance initial adhesion of microorganisms, make attachment more predictable or selective for particular minerals by tailoring culture growth conditions. The impact of growth history on subsequent cell surface properties and attachment efficiencies is the topic of ongoing research. It is recommended that the pH range of the zeta potential study be increased and that the surface properties (both surface charge and hydrophobicity) be monitored throughout the mineral adaptation process.

For the study investigating the attachment of the thermophile, *S. metallicus* to sulfide mineral concentrate systems at ambient temperatures, no explicit attachment trend was evident. Both ore free and chalcopyrite mineral-adapted *S. metallicus* cultures washed out after 1.5 residence times (90 minutes) with the exception of mineral-adapted *S.metallicus* contacted with pyrite where washout was only observed after two residence times (120 minutes). The wash out occurred more rapidly for ore-free cultures as mineral-adapted cultures exhibited high initial cell retention. This may be indicative of enhanced or selective attachment of mineral-adapted cultures relative to ore-free cultures. The highest overall attachment was observed for chalcopyrite mineral-adapted cultures to pyrite at 28 ± 9.9 %, suggesting that mineral-adapted *S. metallicus* exhibit an enhanced affinity for attachment to pyrite, and attach preferentially to pyrite over chalcopyrite. Growth of cultures on sulfide mineral enhances the extent of subsequent attachment. While data are relevant to the initial phase of a heap bioleach operation where temperatures are ambient, they underestimate the maximum levels of attachment attainable for *S. metallicus* due to sub-optimal thermal conditions influencing microbial activity. It is recommended that the experiments for the chalcopyrite mineral system be repeated under temperature conditions optimal for *S. metallicus.*

For the low-grade chalcopyrite mineral system, mineral adaptation of cultures to pyrite concentrate resulted in enhanced attachment, consistent with observations for the sulfide mineral concentrate systems. For the control mineral quartz, the opposite was evident. A decrease in attachment efficiency was found relative to the ore-free cultures. These results are explicable through electrostatic and hydrophobic interactions between the microbial and mineral surfaces under the conditions of this experiment.

For mineral-adapted *A. ferrooxidans,* attachment efficiencies of 25 ± 7.9 and 23 ± 11.3 % were observed to low-grade ore and quartz respectively, with attachment efficiencies of 50 ± 4.1 and 25 ± 7.2 % to each respective mineral observed for mineral adapted *L. ferriphilum.* For ore-free cultured *A. ferrooxidans,* attachment efficiencies of7 ± 4.7 and 35 ± 0.8 % were observed to low-grade ore and quartz, with 26 ± 1.5 and 51 ± 4.5 % observed to each respective mineral for *L. ferriphilum*. Highest levels of attachment were observed for attachment of mineral adapted microorganisms to the pyrite mineral concentrate system, followed by chalcopyrite mineral concentrate, with the least extensive attachment observed to low-grade chalcopyrite and quartz. Thus, selective attachment was demonstrated using the packed column reactor system, with attachment observed to be preferential to sulfide minerals over the control mineral quartz.

Chapter 6: Biofilm reactor attachment study

6.1 INTRODUCTION

In Chapter 4, trends in attachment of microorganisms to mineral surfaces in batch agitated systems were investigated. Although the employment of this approach may provide insight into the trends of microbial attachment to sulfide minerals, it was concluded that the results were not expected to represent attachment dynamics adequately in a heap environment. In order to attain information on attachment under hydrodynamic conditions more representative of bioleach heaps, the study was extended to continuous flow through systems in packed column reactors, described in Chapter 5. Subsequently attachment trends, and factors influencing these, were elucidated and discussed with special regard to the initial adhesion mechanism under heap-like conditions. These observations and discussions were limited to bulk trends observed using pure cultures and mineral coated glass beads. To supplement these investigations, a novel *in situ* approach was developed for visualising and investigating microbial attachment to massive and low-grade chalcopyrite containing mineral ore sections.

This was carried out through the use of a biofilm reactor which consisted of a circular vessel (10 cm outer diameter) in which a section of mineral ore could be housed and irrigated with a model raffinate at linear velocities expected under heap conditions. The thinly sectioned ore was mounted onto glass microscope-slides and mineralogically mapped using ore microscopy and photographic analysis. These low-grade (gangue containing) chalcopyrite mineral ore sections were also used to study microbial attachment following positioning of the mineral ore section, the reactor was sealed with a perspex lid and housed within a laminar flown fume hood to minimise contamination, with continuous irrigation of the mineral surface provided. Once colonised, the thin sections were removed from reactor and the attached cells visualised through the use of fluorescence and ore microscopy techniques.

Experiments to investigate the trends in attachment and colonisation of pure cultures of *A. ferrooxidans*, as well as a mixed consortium of *A. ferrooxidans* and *L. ferriphilum* to massive chalcopyrite thin sections were conducted. Results are presented and discussed in Section 6.2. Mineralogical mapping of low-grade thin sections as well as a study investigating the attachment of a mixed consortium of *A. ferrooxidans* and *L. ferriphilum* to low-grade chalcopyrite mineral containing ore is presented and discussed in Section 6.3. Conclusions are drawn in Section 6.4.

6.2 BIOFILM REACTOR STUDIES USING A MASSIVE CHALCOPYRITE SECTION

6.2.1 Biofilm reactor studies using a massive chalcopyrite section: Results

In order to demonstrate the potential of the experimental technique, experiments were conducted using pure chalcopyrite thin sections. The biofilm reactor was operated as a closed recycle system at a flow rate of 300 µl min^{-1} for the duration of the experiment, which ranged from 72 to 96 hours. The second experiment presented a mixed population containing an equal proportion of *A. ferrooxidans* and *L. ferriphilum*. The reactor was operated as a closed recycle system for the inoculation period after a continuous flow through system was established at 60 µl min^{-1} (7.8 x10^{-5} m s^{-1}). For the pure culture study, the attached population was visualized using 4’, 6-diamidino-2-phenylindole (DAPI) only, after 72 and 96 hours. Micrographs of three different regions on the surface of two different thin sections are presented in Figure 6.1 (a) and (b) for each contact period respectively.

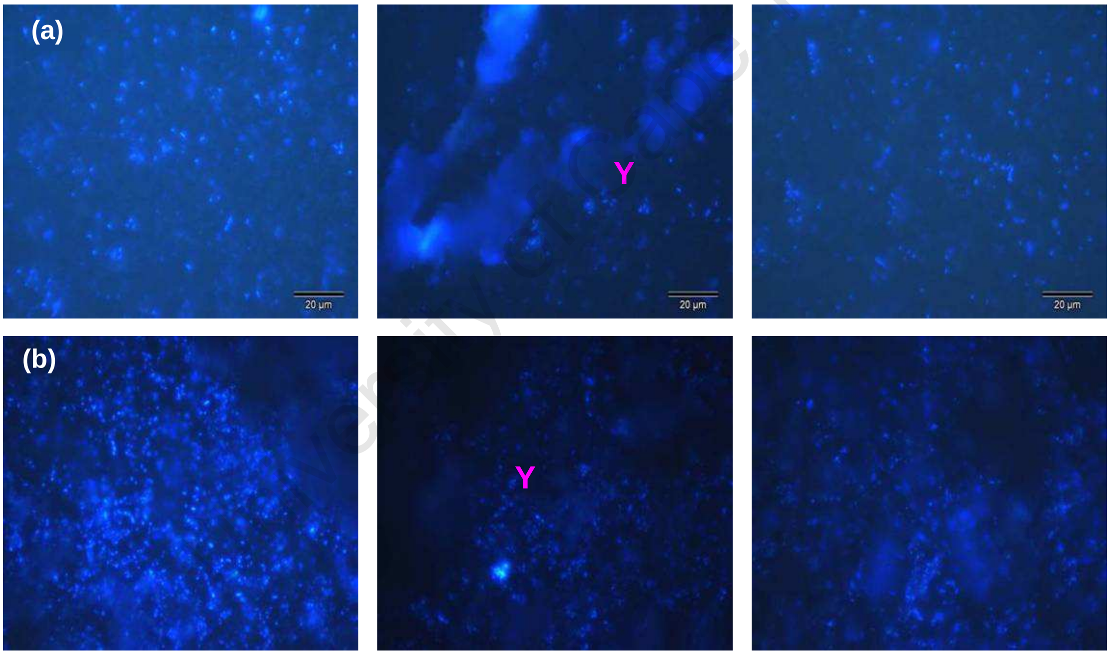

Figure 6.1: DAPI fluorescence of an *A. ferrooxidans* population attached to the surface of two separate 100 µm unpolished massive chalcopyrite thin sections after a **(a)** 72 and **(b)** 96 hour contact period of cells with the mineral in the biofilm reactor. The reactor was operated as a closed recycle system at a flow rate of 300 µl min^{-1}. Attached populations were visualised using an Olympus Epifluorescent microscope under 1000x magnification and UV excitation of the fluorescent stain. Scale bars represent 20 µm.

For both the 72 and 96 hour contact periods, a mono-layered expanse of cells, distributed across the most of the surface of the section was visible, with cells concentrated at regions displaying surface defects (rough edges, grain boundaries and cracks in the surface) labelled Y in Figure 6.1. The attached population was observed to be denser after 96 hours relative to the 72 hour contact period with individual cells still visible for both contact periods under these experimental conditions. The DAPI staining technique was improved between the observations at 72 and 96 hours in this experiment. The lysozyme cell permeabilisation step and the ethanol dehydration step were incorporated into the staining procedure to improve penetration of DAPI into cells and thereby enhance complexation with DNA. A marked improvement in the quality of the fluorescence images is thus seen in Figure 6.1 (b) relative to Figure 6.1 (a). These improvements were maintained for all subsequent microscopy.

The study was extended to consider attachment of a mixed microbial inoculum consisting of equal proportions of *A. ferrooxidans* and *L. ferriphilum* to massive chalcopyrite. For the inoculation period the reactor was run as a closed recycle at a flow rate of 60 μl min^{-1} (7.8 $x10^{-5}$ m $s^{-}1$) for a contact period of 20 hours, whereafter the reactor was operated as a continuous flow through system. DAPI was used as a universal fluorescent counter-stain as DAPI complexes with AT-rich sequences of double stranded DNA and will be taken up by all microbial species present. A target fluorescent stain, using FISH probe (LF581), specific for the *Leptospirillum* genus was employed for identification and differentiation of *L. ferriphilum* from *A. ferrooxidans*. The DAPI and corresponding FISH fluorescence results for attachment of a mixed culture of *A. ferrooxidans* and *L. ferriphilum* are presented in Figures 6.2 and 6.3.

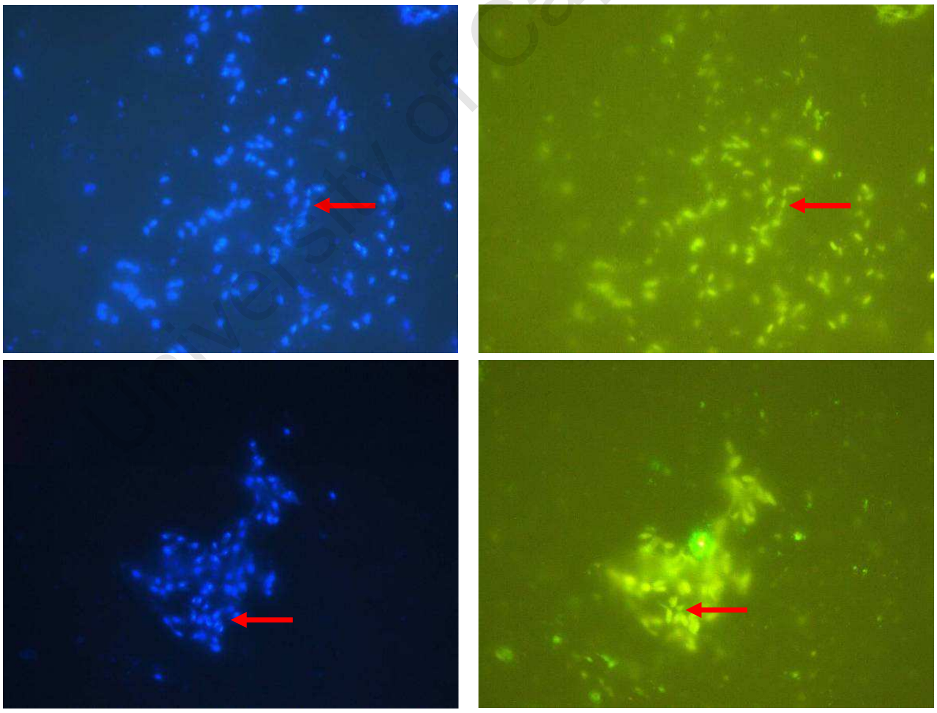

Figure 6.2: Attachment of a mixed consortium of *A. ferrooxidans* and *L. ferriphilum* to an unpolished, 100 μm massive chalcopyrite thin section. The biofilm reactor was operated as a closed recycle system for the 20 hour inoculation period at a flow rate of 60 μl min^{-1}, whereafter it was operated as a continuous flow through system. DAPI fluorescence of the attached population is presented in **(a)** with the corresponding FISH fluorescence using the LF581 probe specific for *Leptospirillum sp* .observed presented in **(b)**.

In several regions, the attached populations were mixed-micro-colonies which consisted of both *A. ferrooxidans* and *L. ferriphilum*. This was apparent due to the fact that cells exhibited green fluorescence with the target stain using FISH probe (LF581) indicating the presence of attached *Leptospirillum* species. The density of the cells exhibiting the target stain fluorescence was less extensive than cells exhibiting fluorescence with the universal DAPI stain which would be exhibited by all attached species. This was indicative of a mixed microbial colony attached to the surface of the chalcopyrite thin section.

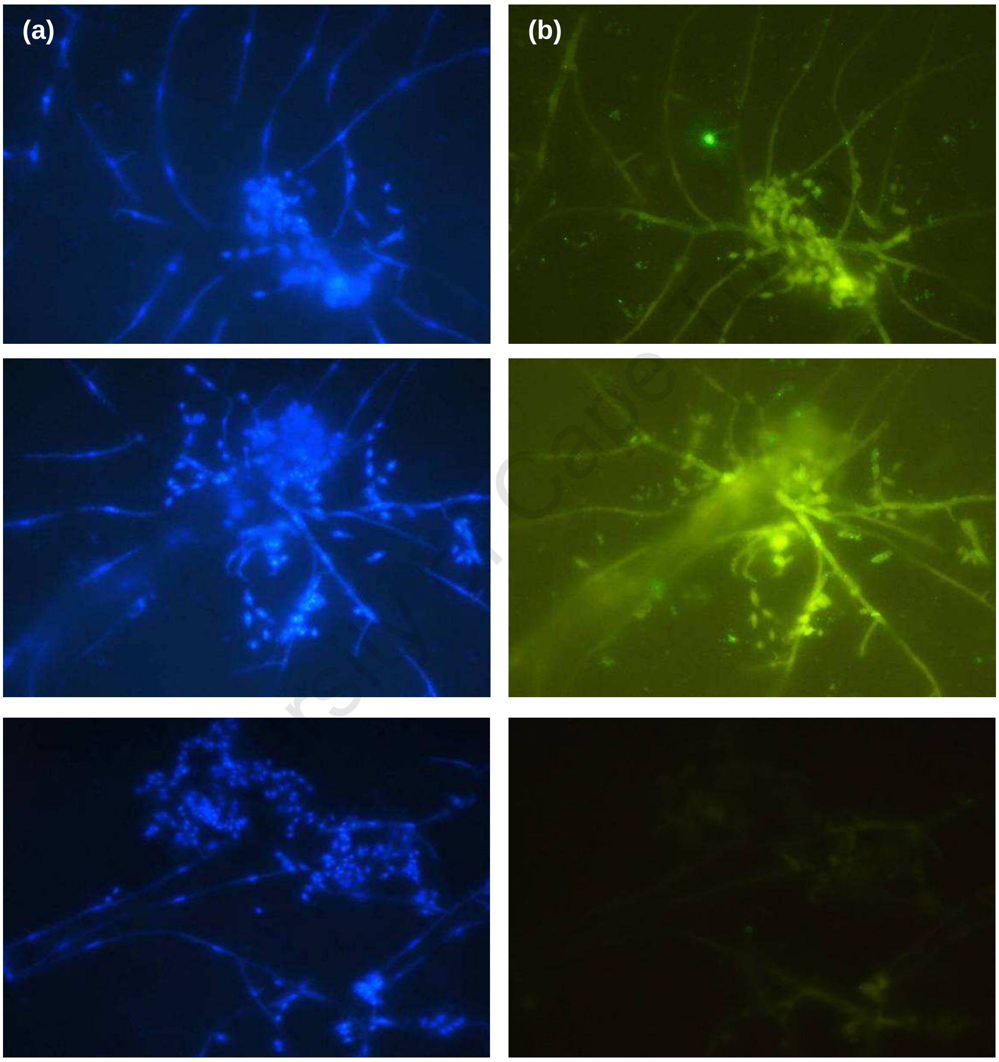

Figure 6.3: Attachment of a mixed consortium of *A. ferrooxidans* and *L. ferriphilum* to an unpolished, 100 µm massive chalcopyrite thin sections. The biofilm reactor was operated as a closed recycle system for the 20 hour inoculation period at a flow rate of 60 µl min^{-1}, whereafter it was operated as a continuous flow through system. DAPI fluorescence of the attached population is presented in **(a)** with the corresponding FISH fluorescence using the LF581 probe specific for *Leptospirillum sp.* observed for the respective region in **(b)**.

In Figure 6.3, no fluorescence was observed using the target (FISH) stain in some regions. This suggests that there were no attached *L. ferriphilum* cells present in these regions. The morphology of the cells attached in these regions was investigated viewing the cells which exhibited universal DAPI counter-stain fluorescence. These cells were larger and rod-shaped, typical of *A. ferrooxidans* whereas *L. ferriphilum* are smaller and exhibit a spiral-like shape. Hence, the absence of very low numbers of *L. ferriphilum* was supported. Alternatively, the image may have been underexposed resulting poorly discernable fluorescence.

No cells were found in the effluent stream by microscopic counting once the reactor was operated as a continuous flow through system. This suggests that all the cells became attached to the surface of the thin section. Alternatively, cell concentration in the effluent stream was below the detection limit (of 10^5 cells ml^{-1}) of the enumeration method used.

6.2.2 Biofilm reactor studies using a massive chalcopyrite section: Discussion

The biofilm reactor approach presented a novel technique for visualising and investigating attachment to mineral (chalcopyrite) ore sections *in situ*. This approach has been successfully employed as a means of investigating attachment and subsequent colonisation of pure and mixed mesophilic cultures, *in situ*, through the use of fluorescence microscopy. Individual attached cells were clearly visible on the surface of the thin section in both pure and mixed cultures through the use of the universal counter-stain (DAPI). The use of fluorescent *in situ* hybridisation (FISH) using the LF581 *Leptospirillum* specific probe provided a sufficient means of identification and differentiation of attached microbial species, as cells which exhibited positive fluorescence using the target probe were clearly discernable. Sterile conditions were maintained throughout the experiment, allowing investigation of the attachment of selected microorganisms.

For pure culture experiments, dense expanses of attached *A. ferrooxidans* populations were seen, with individual cells clearly discernable. This was indicative of the development of an actively growing mono-layer biofilm of *A. ferrooxidans* on the surface of the massive chalcopyrite section. These findings are in accordance with Kinzler *et al.* (2003), Harneit *et al.* (2005), Sand *et al.* (2006), Gerhke *et al.* (2001) and Sanhueza *et al.* (1999) where the development of biofilms by bioleaching microorganisms was demonstrated. By contrasting the results presented in Figure 6.1 and 6.2, it is evident that similar attachment trends were observed for experiments conducted using mixed populations relative to the pure culture experiments. Individual attached cells were clearly visible using DAPI staining techniques. These clustered predominantly in regions of surface defects. The density of the attached population was more extensive for the pure culture experiments. For these studies, flow rates of 300 µl min^{-1} used corresponded to a residence time 1.1 hours. Thus, sampling from the closed recycle system at 72 and 96 hours corresponded to 66 and 87 residence times. The exact time taken for the onset of a firmly attached microbial population could not be determined under these conditions.

For the mixed culture studies, the flow rate of 60 µl min^{-1} (7.8 x10^{-5} m s^{-1}) which corresponded to a residence time of 5.6 hours. In addition the duration of the experiment was only 48 hours, the first 20 of which was operated as a closed recycle system used for inoculation. Thus effective contacting of microorganisms with the mineral surface and the establishment of a firmly attached colony occurred after 3.6 residence times during the inoculation period. The decrease in the density of the attached microbial population observed can be attributed to the lower cell concentration and the contact time in experiments using mixed cultures relative to the pure culture experiments.

For both pure and mixed culture experiments, tightly clustered micro-colonies were observed predominantly in regions where visible site defects were abundant (Figure 6.1 and 6.3). This is in accordance with literature reports by Gehrke *et al.* (1998), Rojas-Chapana *et al.* (1998) and Harneit *et al.* (2005). However, attached micro-colonies were not limited to these sites as attached cells were also observed to be dispersed sparsely. Andrews (1988), in Gehrke *et al.* (1998 and 2001), suggested that sulphur atoms concentrate at grain boundaries and surface imperfections making these locations more favourable as sites of attachment. Rojas-Chapana *et al.* (1998) proposed that nutrient concentration gradients were generated at pyrite fragmentation sites, which attracted the settlement of microbes at these specific sites over others.

6.3 BIOFILM REACTOR STUDIES USING A LOW-GRADE CHALCOPYRITE SECTION

6.3.1 Mineral identification and mapping of low-grade chalcopyrite thin sections

Ore microscopy exploits the optical properties of various minerals under reflected and transmitted light in polarised and cross polarised planes in order to identify minerals present. Ore microscopy, together with mineralogical information provided, was used to characterise ore samples and identify minerals present. Important properties which guide the identification of specific minerals include the physical properties of the mineral as well as optical properties. Optical properties refer to properties such as refractive index, luminescence and colour, as well as pleochroism, isotropism or anisotropism, birefringence, internal reflectance and twinning, all of which may be determined using ore microscopy (Rogers 1937). To demonstrate the detail of the methodology employed in mineral identification and mineralogical mapping, the results observed for the mapping of copper containing ore from two mines; Kennecott and Escondida, are presented and contrasted. Microbial attachment studies were conducted using Escondida ore. The use of Kennecott ore was employed in development and validation of the robustness of the mineralogical mapping technique.

6.3.1.1 Mineralogical mapping and mineral identification of Kennecott ore

Low-grade chalcopyrite mineral containing ore samples were cut into 60 and 100 µm thin sections, and mounted onto glass slides using an epoxy resin (Department of Geology, University of Cape Town). The 60 µm thin sections were polished for the purposes of mineral identification and scanned to create an electronic photographic data base. The surface of the thin section is divided into various regions, which were transcribed onto the scanned images, in order to systematically generate a mineralogical map of the surface of the sample. Two such thin sections, labelled K2 and K3, constructed from Kennecott ore are presented in Figure 6.4. For this particular study, the rationale regarding the subdivision of the surfaces was to consider regions containing sulfide minerals of interest as well as a large representation of the gangue mineralogy present in order to demonstrate the mineral identification technique and optimise it for subsequent attachment studies. For attachment studies, the rigor involved, and detail regarding the sectioning of the surface of the sample was increased to allow for adequate and sufficient coverage of the entire surface.

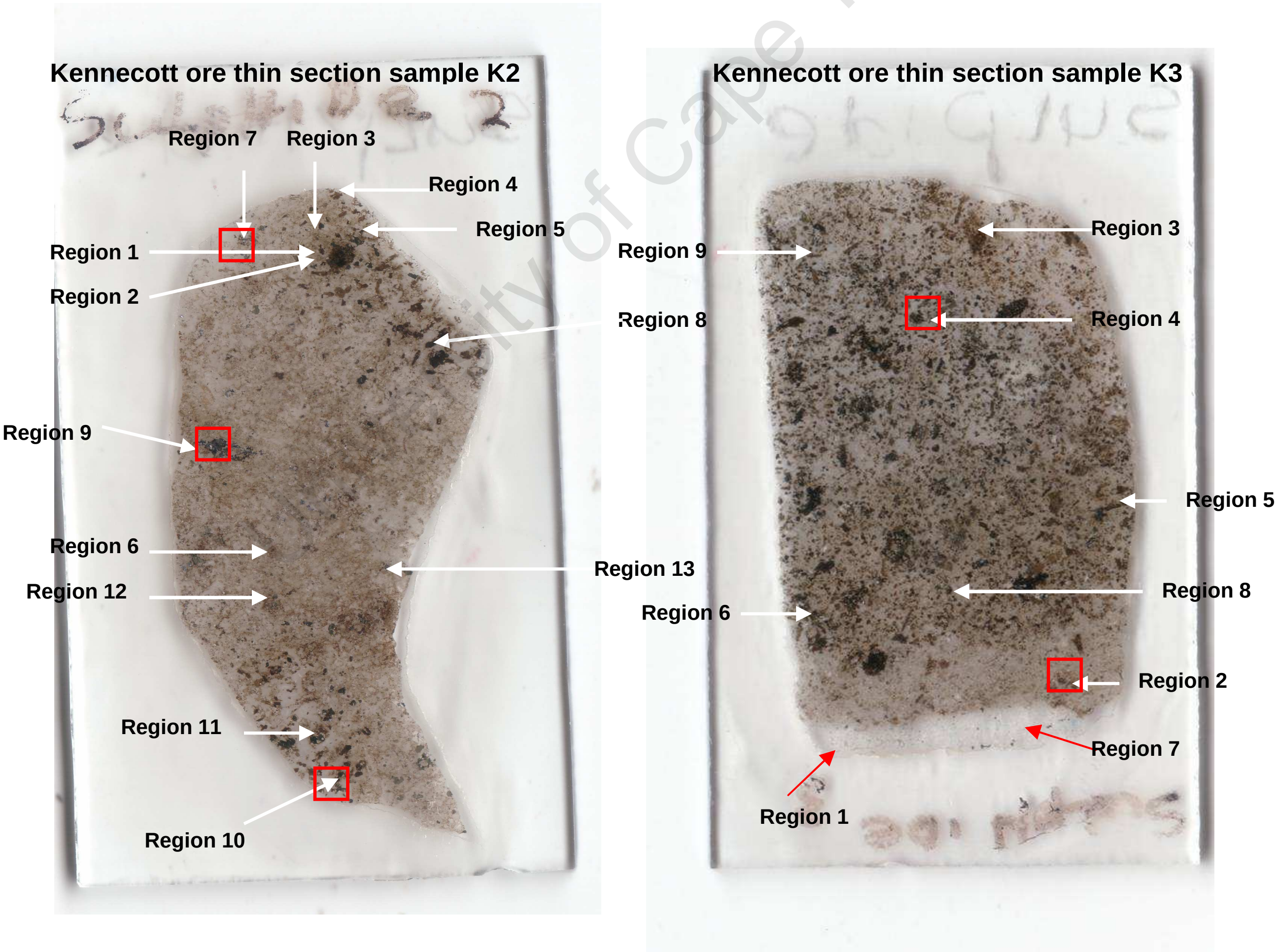

Figure 6.4: This figure depicts scanned images of the Kennecott copper ore thin section samples K2 (left) and K3 (right) and division of the mineral surface into various regions for the generation of a mineralogical map through the use of ore microscopy and photographic analysis.

The identity of the predominating sulfide and gangue minerals present was determined through comparison of characteristic optical properties under reflected and transmitted light as summarised in Table 6.1. Photographs of the different regions on the surface were taken using various magnifications (5 x, 10 x and 50 x objectives), which allowed for good coverage of mineral surface with the spatial locale of minerals identified noted.

Upon analysis of Figure 6.5, the presence of three sulfide minerals were predominant; namely pyrite, chalcopyrite and molybdenite. These were evident through comparison of the characteristic optical properties under reflected and transmitted light as summarised in Table 6.1. Sulfide minerals reflect light and thus pyrite, chalcopyrite and molybdenite appeared pale yellow, brassy yellow or light grey under reflected light respectively (Anthony *et al.* 1990). Sulfide minerals are isotropic opaque minerals (Anthony *et al.* 1990) and do not transmit light when viewed using transmitted light microscopy, thus all sulfide minerals appeared black under transmitted light. This is evident in Figure 6.5 (a) to (c) where pyrite, chalcopyrite and molybdenite was denoted X, Y and Z respectively, with sulfide collectively labelled S.

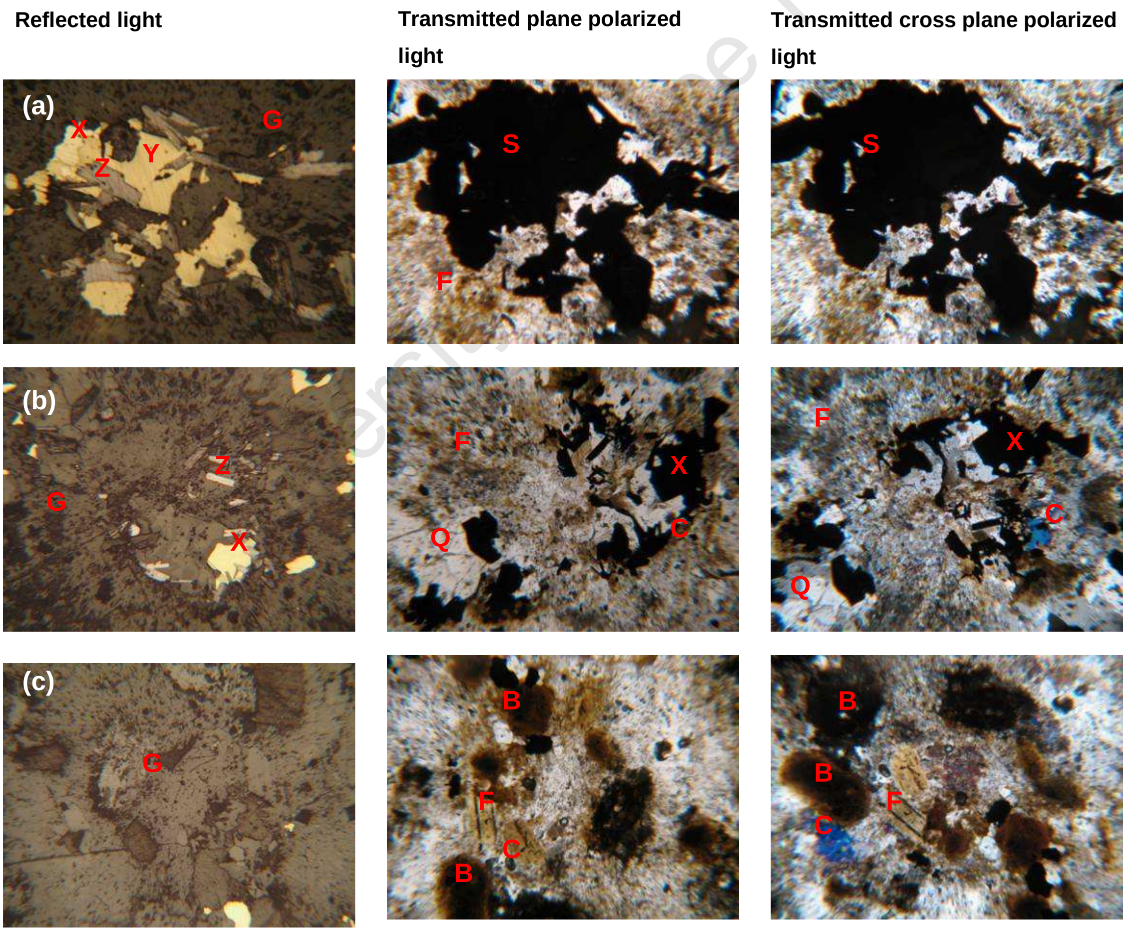

Figure 6.5: (a) Region 9, **(b)** Region 7 and **(c)** Region 10 on sample K2. Images were viewed under reflected light, transmitted plane polarised and transmitted cross polarised light from left to right respectively, using the 5 x objective on a Zeiss polarising light microscope. The field of view equated to 2.5 mm. Pyrite was denoted X, chalcopyrite denoted Y, molybdenite denoted Z, gangue minerals collectively denoted G, feldspars collectively denoted F, quartz denoted Q, calcite denoted C and biotite denoted B.

Table 6.1: The major sulfide and gangue minerals present in the Kennecott ore sample, and the associated characteristic optical properties used for their identification is presented. (Rodgers 1937, Anthony *et al.* 1990 and Deer *et al.* 1992)

Sulfide minerals

Mineral	**Mineral formulae**	**Characteristic optical properties**	
		Reflected light	**Transmitted light**
Chalcopyrite	$CuFeS_2$	Brassy yellow	Black
Pyrite	FeS_2	Pale yellow	Black
Molybdenite	MoS_2	Light grey	Black

Gangue minerals

Mineral	**Mineral formulae**	**Reflected light**	**Transmitted light**	
			PPL	**XPL**
Quartz	SiO_2	Dull brown to grey (does not reflect light)	Colourless to milky or white in thin sections	Exhibits weak birefringence (0.009) Isotropic Light to darker greys and white interference colours
Plagioclase	(Na,Ca) Al(Al,Si) Si_2O_8	Dull brown to grey (does not reflect light)	White to light-grey and colourless, may display lamellar twinning	Exhibits weak birefringence (0.007- 0.013)
Orthoclase	$KAlSi_2O_8$	Dull brown to grey (does not reflect light)	Colours may vary from white to colourless, cream to tan, may display cross hashed twinning May exhibit cross hash twinning	Exhibits weak birefringence (0.006-0.010) Extinction angle occurs between 5 to 19º
Biotite	$K(Mg,Fe,Al)_3$ $AlSi_3O_{10}(OH)_8$	Dull brown to grey (does not reflect light)	Black or dark brown	Strong birefringence (0.028-0.08) Exhibits pleochroism
Chlorite	$(Cl, Mg,Fe,Al)_6$ $AlSi_3O_{10}(OH)_2$	Dull brown to grey (does not reflect light)	Green of various tints, varying from almost white to almost black.	Exhibits weak to moderate birefringence (0.007-0.015) With yellow to green to very dark interference colours.
Calcite	$CaCO_3$	Dull brown to grey (does not reflect light)	Clear or white, to tan or grey. May display lamellar twinning.	May be tinted many colours and is anisotropic. Displays rainbow pleochroism. Exhibits strong birefringence 0.172-(0.190).
Amphibole (Hornblend)	$mCa(Mg,Fe)_3$ $(SiO_3)_4$ + $n(Al,Fe)(F,OH)$ SiO_3	Dull brown to grey (does not reflect light)	Green to brown	Exhibits birefringence (0.022 – 0.027) With moderate to strong, white and/or yellow interference colours. Extinction at 10 to 20˚

Plane polarised light **(PPL)**, cross polarised light **(XPL)**

Large grains of chalcopyrite locked within gangue minerals were evident, with chalcopyrite grains disseminated throughout the sample (Titley *et al.* 1966). The observation of chalcopyrite being locked within gangue minerals highlights the difficulty of leaching low-grade ore bodies as gangue dissolution needs to be extensive for chalcopyrite dissolution to occur effectively. This also highlights the importance of mineralogy in increasing our understanding and prediction of heap leaching both chemically and microbially.

Through interrogation of Figure 6.5 (a) to (c), and Table 6.1, the predominating gangue minerals identified were: quartz denoted Q; the feldspars plagioclase and orthoclase, denoted F; calcite, denoted C and biotite, denoted B. The gangue minerals present do not reflect light and thus appeared dull grey or brown under reflected light. Calcite, evident in Figure 6.5 (b) and (c), exhibited a “rainbow” of interference colours under cross polarised light characteristic of this anisotropic, pleochroic mineral which exhibits strong birefringence (Rogers 1937, Deer *et al.* 1992). The feldspars denoted F in Figure 6.5 (c) were most likely plagioclase owing to the characteristic lamellar twinning exhibited and isotropic properties under cross polarised transmitted light (Rogers 1937, Deer *et al.* 1992).

The images observed for Regions 2 and 4 of sample K3 are shown in Figure 6.6. Upon interrogation of these images and information summarised in Table 6.1, the minerals present in sample K3 could be identified. The presence of pyrite was evident in Figure 6.6 (b). The pyrite grain characteristically appeared pale yellow under reflected light and black under transmitted light. The presence of quartz and feldspars were evident in Figures 6.6 (a). The presence of calcite was apparent in Figure 6.6 (b) due to the characteristic “rainbow” interference colours exhibited by this anisotropic, pleochroic mineral. The presence of chlorite was also evident and appeared greenish brown under transmitted plane and exhibited dark interference colours under cross polarised light, seen in Figure 6.6 (a).

The predominating sulfide and gangue minerals identified through the use of ore microscopy and the inherent optical properties of minerals under reflected and transmitted light can be corroborated with mineralogical information provided for the Kennecott ore sample. Quantitative Evaluation of Minerals by Scanning electron microscopy (QEMSCAN) was conducted on the Kennecott ore sample (D. Latti of Rio Tinto Technical services, Melbourne) and the results made available (AMIRA P768A, CSIRO Minerals 2006). The mineralogical composition of the Kennecott ore sample provided by QEMSCAN analysis is presented in Table 6.2. From the data presented in Table 6.2, it can be seen that the predominating gangue minerals are quartz and silicates, like the feldspars plagioclase and orthoclase as well as the mica biotite. The predominating sulfide minerals are chalcopyrite and pyrite. This agrees well with the results observed using ore microscopy. However, the presence of molybdenite was identified using ore microscopy. This sulfide mineral was not represented in the QEMSCAN analysis.

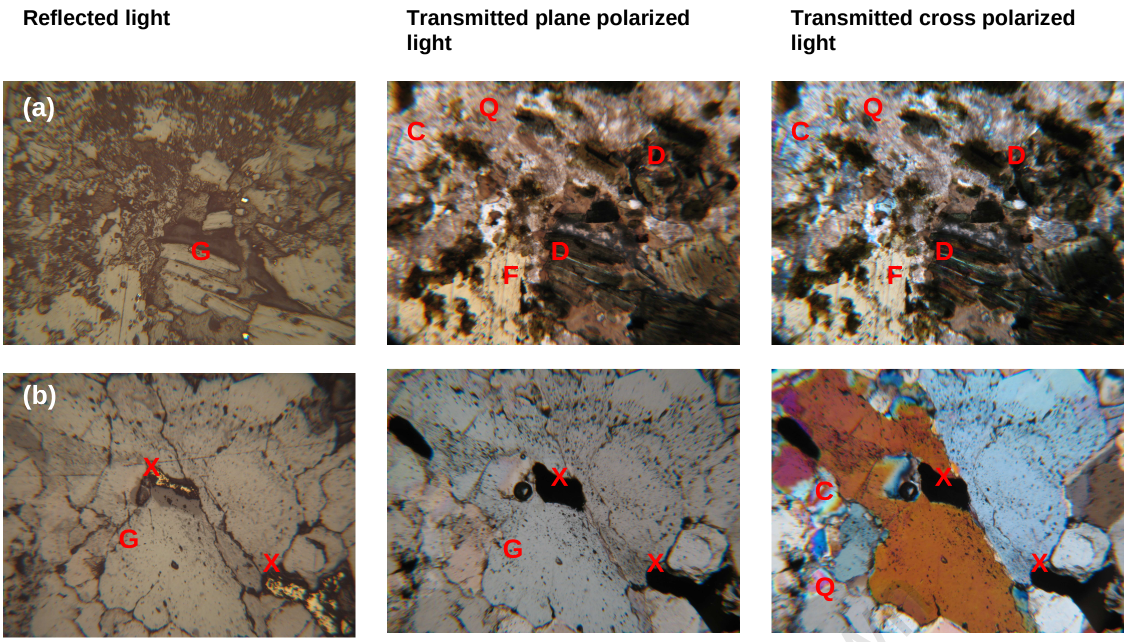

Figure 6.6: (a) Region 4 and **(b)** Region 2 on sample K3 viewed under reflected, transmitted plane polarised and transmitted cross polarised light from left to right respectively. Images were viewed using the 5 x objective (Field of view equates to 2.5 mm) on a Zeiss polarising light microscope. Pyrite was denoted X, chalcopyrite denoted Y, molybdenite denoted Z, gangue minerals denoted G, feldspars denoted F, quartz denoted Q, calcite denoted C, biotite denoted B and chlorite denoted D.

Table 6.2: Mineralogical (QEMSCAN) information provided for the Kennecott ore sample. These data were provided as the percentage abundance in various size fractions. The upper and lower percentages are presented in the table. Size fractions ranged from +13 mm to -1.18 mm.

Mineral	Mineral formulae	% Mineral abundance per size fraction
Chalcopyrite	$CuFeS_2$	1 - 2.6
Pyrite	FeS_2	0.1 - 0.7
Quartz	SiO_2	15.3 – 28.8
Plagioclase	$(Na,Ca)\ Al(Al,Si)\ Si_2O_8$	4.9 – 10.3
Orthoclase	$KAlSi_2O_8$	32.2 – 41.9
Biotite	$K(Mg,Fe,Al)_3\ AlSi_3O_{10}(OH)_8$	16.4 – 22.7
Chlorite	$(Mg,Fe,Al)_6\ AlSi_3O_{10}(OH)_2$	3.0 – 3.6
Apatite	$Ca_5(PO_4)\ (Cl,F,OH)$	1.7 – 2.1
Calcite	$CaCO_3$	0.2 – 0.7

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6.3.1.2 Mineralogical mapping and mineral identification of Escondida ore

Ore microscopy was used to map thin sections of low-grade ore from Escondida containing chalcopyrite (CH-32-Type B). As described previously, the ore was sectioned into 60 µm or 100 µm thin samples and mounted onto glass slides. The thinner sections were polished and used for mineral mapping and identification. A scanned image of such a sample, labelled C, is presented in Figure 6.7. Thicker sections were left unpolished and used for the purpose of the biofilm attachment study.

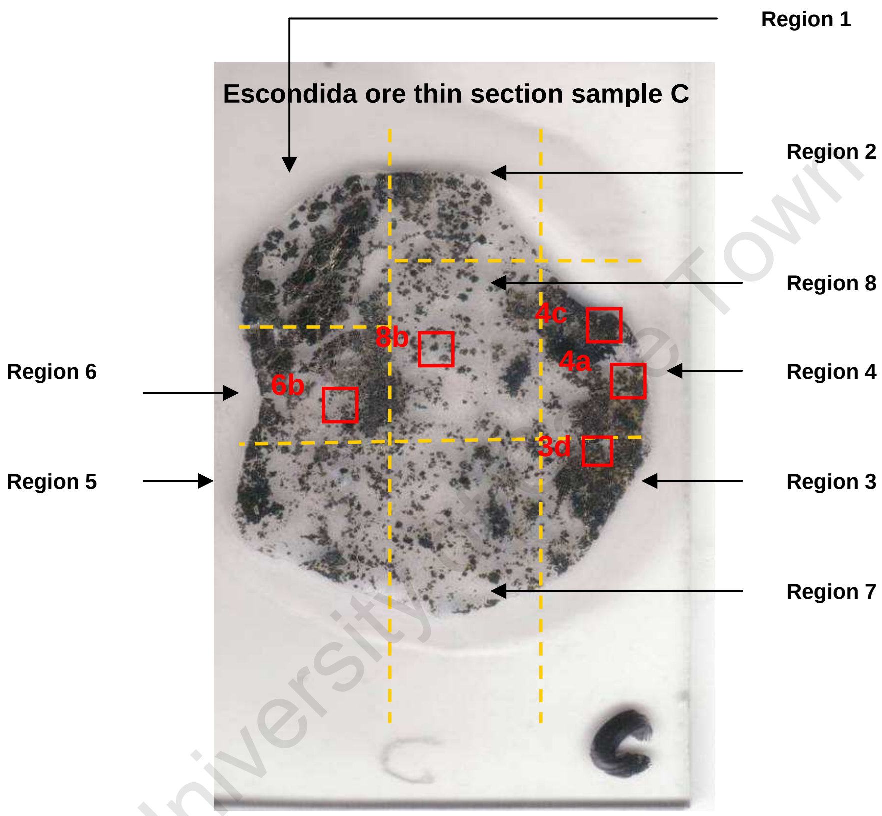

Figure 6.7: Mapped 60 µm polished thin section (CH32-Type B ore) sample "C". The sample was divided into regions as depicted.

A summary of the mineralogical information provided (courtesy of BHP Billiton), as well as a summary of the characteristic optical properties used for mineral identification under reflected and transmitted light was compiled and is presented in Table 6.3. Photographs of the various regions on the surface of the samples were taken and the identity of the predominating sulfide and gangue minerals present determined through comparison of characteristic optical properties under reflected and transmitted light as summarised in Table 6.3. The spatial locale of minerals identified was noted. The results of the images captured for Regions 4a, 4c, 6b and 8b, demarcated with red squares in Figure 6.7, are depicted in Figure 6.8.

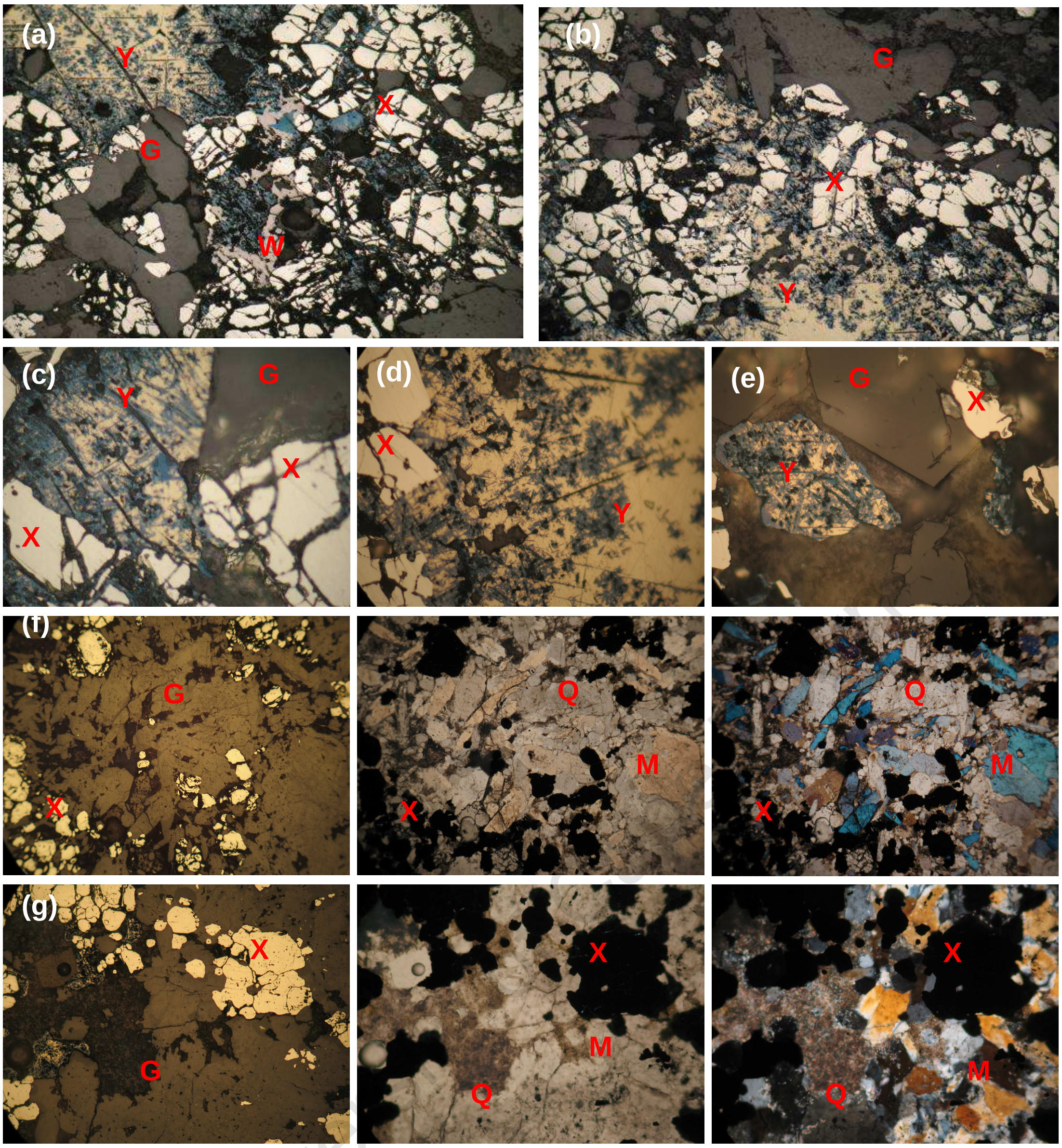

Figure 6.8: Photographs corresponding to various regions on the surface of a low-grade chalcopyrite containing mineral thin section labelled sample C (Escondida, CH32-Type B). **(a)** Region 4a under (**RL,** 10 x objective); **(b)** Region 4a (**RL** 5 x objective); **(c)** Region 4a (**RL** 50 x objective); **(d)** Region 4c (**RL** 50 x objective); **(e)** Region 4c (**RL** 50 x objective); **(f)** Region 8b using the 5 x objective and **(g)** Region 6b using the 10 x objective, under **RL**, **TL-PPL** and **TL-XPL** respectively from left to right. Pyrite denoted with X, chalcopyrite exhibiting covellite intergrowth denoted Y, chalcocite denoted W, gangue minerals collectively denoted G, quartz denoted Q and muscovite denoted M. (reflected light = **RL**, transmitted light = **TL**, plane polarised light = **PPL**, cross polarised light = **XPL**)

Table 6.3: Summary of the predominating sulfide and gangue minerals present (wt %) in the Escondida CH-32-Type B ore (courtesy of BHP Billiton), as well as a summary of the optical properties used for mineral identification under reflected and transmitted light using ore microscopy (Rodgers 1937, Anthony *et al.* 1990 and Deer *et al.* 1992). The complete mineralogical composition of the Escondida CH-32-Type B ore is provided in Appendix E.

Mineral	**Chemical composition**	**wt%**	**Characteristic optical properties**		
			Reflected Light	**Transmitted Light**	
Major sulfide minerals					
Pyrite	FeS_2	4.0	Cream to pale yellow	Black	
Chalcopyrite	$CuFeS_2$	0.5	Brassy yellow	Black	
Covellite	CuS	0.3	Green intergrowth on chalcopyrite surface	Black	
Chalcocite	Cu_2S	0.2	Pale grey	Black	
Major gangue minerals				**PPL**	**XPL**
Quartz	SiO_2	44.8	Dull brown grey (does not reflect light)	Colourless to milky in thin sections	Isotropic Exhibits weak birefringence (0.009) with light to darker greys and white interference colours
Muscovite	$KAl2(Si_3Al)O_{10}(OH;F)_2$	28.6	Dull brown grey (does not reflect light)	Colourless in thin section, may appear tinted in hues of grey or brown	Anisotropic Exhibits high birefringence (0.035 - 0.042) with interference colours ranging from the violet through to red spectrum

Plane polarised light (**PPL**), Cross polarised light (**XPL**)

Upon examination of the results in Figure 6.8 and Table 6.3, the predominating sulfide minerals present were identified as pyrite, chalcopyrite which exhibited covellite intergrowth, and chalcocite. The presence of chalcopyrite exhibiting covellite intergrowth was evident, and and was denoted “Y” in Figure 6.7 (a) to (e). Chalcopyrite exhibited a characteristic brassy yellow hue under reflected light with green covellite intergrowth observed. Chalcopyrite is commonly associated with covellite (Anthony *et al.* 1990). Chalcopyrite grains were relatively large and in some instances appeared to be locked within a gangue mineral matrix, as observed in Figure 6.7 (e). The presence of chalcocite was also observed, and was denoted “W” in Figure 6.7 (a). The presence of pyrite grains was evident in Figure 6.7 (a) to (g) and was denoted “X”. The pyrite grains appeared pale yellow under reflected light, relative to the brassy yellow colour observed for chalcopyrite grains, and appeared black under transmitted light.

Pyrite grains exhibited a distinct brecciated texture and were disseminated throughout the sample. In some instances, the pyrite grains were observed to be encased in a matrix of chalcopyrite, observed in Figure 6.7 (c) and (d). Chalcopyrite and associated covellite were less abundant than the pyrite crystals, as per visual inspection, with chalcocite observed to be the least prevalent sulfide mineral. This was consistent with mineralogical information, summarised in Table 6.3 and Appendix E, where greatest proportion of sulfide minerals present in the ore sample was reported to be pyrite (4 wt %), followed by chalcopyrite (0.5 wt %), covellite (0.3 wt %) and chalcocite (0.2 wt %).

Predominating gangue minerals present were identified as quartz and muscovite. Quartz and muscovite were identified using optical properties under transmitted light (Table 6.3). Quartz exhibited a creamy or milky colour under transmitted, plane polarised light, which then appeared to change to different hues of grey under transmitted cross polarised light seen in Figure 6.8 (a). This is characteristic for quartz which is an isotropic mineral with weak birefringence exhibiting grey and white interference colours under cross polarised transmitted light (Rogers 1937, Deer *et al.* 1992), evident in Figure 6.8 (f) and (g). Muscovite appeared cream to tan under plane polarised transmitted light. However, under cross polarised light, muscovite exhibited characteristic strong birefringence with bright pastel colours of blue and yellow displayed at different orientations of the thin section under cross polarised transmitted light. These characteristic interference colours, (Rogers 1937, Deer *et al.* 1992, Dutch S., http://www.uwgb.edu/DutchS/petrolgy/MUSCOV.HTM), are evident in Figure 6.7 (f) and (g). The predominating gangue minerals present identified using ore microscopy and visual inspection was in accordance with mineralogical information supplied, where the greatest proportion of gangue was constituted by quartz (44 wt %), followed by muscovite (28 wt %).

6.3.2 Biofilm reactor studies using low-grade chalcopyrite: Results

Once the thin sections were effectively mapped, the biofilm attachment studies were conducted. The sections were placed in a biofilm reactor and inoculated with a 20 ml suspension of a mixed consortium of *A. ferrooxidans* and *L. ferriphilum* containing 5 x 10^5 cells ml^{-1} per microorganism. The reactor was operated as a closed recycle for the duration of the inoculation period of 20 hours. Subsequently the reactor was operated as a continuous flow through system at a flow rate of 60 µl min^{-1} (7.8 x10^{-5} m s^{-1}). The residence time was calculated to be 5.6 hours at this flow rate. The experiment was operated for 48 hours. Once colonised, the thin section was removed from reactor and the attached cells visualised through the use of fluorescence and ore microscopy techniques. The spatial distribution of the attached microorganisms, relative abundance and spatial locale of the attached microorganisms was assessed.

In attachment studies using low-grade chalcopyrite (Escondida CH-32-Type B) mineral containing thin section (sample C), the observation of attached microbial species in various regions on the surface of the thin section, through the visualisation of DAPI and corresponding FISH fluorescence is presented in Figure 6.9 and Figure 6.10. The mineralogical map of sample C is reproduced showing regions on the surface of the thin section corresponding to the results presented in Figures 6.9 and 6.10.

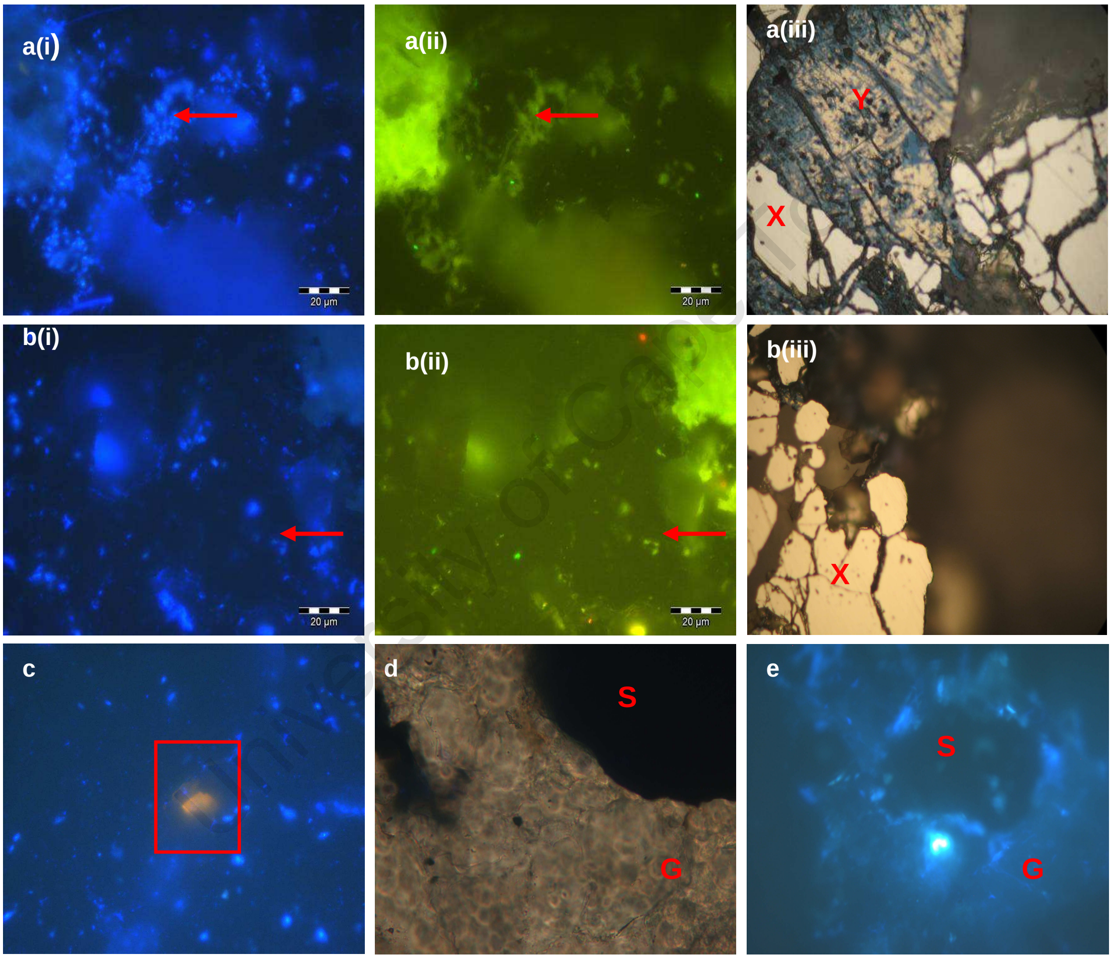

Figure 6.9: Biofilm reactor attachment of *A. ferrooxidans* and *L. ferriphilum* to the surface of a low-grade chalcopyrite containing mineral thin section, depicting **(i)** DAPI fluorescence, **(ii)** corresponding targeted FISH fluorescence, (1000 x magnification, Olympus microscope), **(iii)** corresponding regions from mineralogical map under reflected light (500 x magnification, Zeiss microscope) for the following regions on the surface of the thin section **(a)** Regions 4a and **(b)** Region 3d. Control images show **(c)** gangue mineral auto-fluorescence, **(d)** Region 8 as observed under phase contrast at 1000 x magnification using (white light source) on the Olympus microscope and **(e)** Region 8 as observed under 1000 x magnification using the UV filter (UV light source).

Upon analysis of the results shown in Figure 6.9, individually attached cells were clearly discernable on the surface of the thin section through positive fluorescence of the universal DAPI stain. These attached populations were either dispersed (seen in Figure 6.9 B) or tightly clustered (evident in Figure 6.9 A) into micro-colonies. From the mineralogical maps constructed, it was observed that micro-colonies of attached cells were clustered in regions where sulfide minerals were abundant, evident upon comparison of the micrograph taken of that particular region under reflected light, shown in Figure 6.9 A (iii) and B (iii). The presence of sulfide minerals namely large chalcopyrite grains exhibiting covellite intergrowth (Y) and smaller pyrite grains (X) were evident in Figure 6.9 (A), with pyrite grains evident in Figure 6.9 (B). Regions on the surface of the thin section corresponding to the results presented in Figures 6.9 and 6.10 are indicated with red squares in Figure 6.7.

The observation of microbial attachment to sulfide minerals in Figures 6.9 a(i) and (ii), and b(i) and b(ii) was also corroborated by the control pictures seen in Figure 6.9 (D) and (E). In Figure 6.9 (D) the surface of the section as depicted through the Olympus microscope using a white light source was observed. Due to the intrinsic optical properties, gangue minerals transmit light and appear translucent while sulfide minerals are opaque, do not transmit light and appear dark (black) when viewed under white light, as observed in Figure 6.9 (D). Figure 6.9 (E) depicts the same region using a UV light source and filter. Sulfide minerals appeared dark with background fluorescence observed in regions where the gangue minerals were present.

The exact location of the attachment evident in Figures 6.9 a(i) and a(ii), and b(i) and b(ii) on the surface of the mineral section depicted in a(iii) and b(iii) could not be conclusively distinguished. This was due to limitations of the microscopes used. Two separate microscopes were employed in this study: a Zeiss polarising light microscope was used for mineral identification and mineralogical mapping, with an Olympus Epifluorescent microscope used for visualisation of attached microbial cells. Due to differences in the range of magnification available on the respective microscopes (a maximum of 500 x and 1000 x magnification for the Zeiss and Olympus microscopes respectively), micrographs with comparable fields of view could not be taken. Thus, the precise location of the attachment, pertaining to the actual mineral grain, could not be conclusively determined. It is recommended that procedures be put in place to make the identification of the special locale of the attached cells precise and rigorous in future studies. This may be conducted through physical etching of grid-lines on the surface of the section and a more extensive compilation of micrographs taken subsequent to attachment.

Mixed micro-colonies of attached microbial populations were evident on the surface of the thin section. This was evident upon the differentiation of attached cells by FISH using the target

Leptospirillum specific probe LF581, indicative of the presence of attached *L. ferriphilum,* observed in Figure 6.9 a(ii) and b(ii) and comparison to DAPI stained cells in Figure 6.9 a(ii) and b(ii). Target probe (FISH) fluorescence exhibited in this study was poor. This may have been due to the background fluorescence caused by gangue minerals present, as observed in the control micrograph in Figure 6.9 a(ii), b(ii) and (e), or due to auto-fluorescence of gangue minerals present, seen in Figure 6.9 c. The latter may be attributed to fluorescence of the gangue mineral apatite. Apatite comprised 0.1 wt % of the CH-32 Type B ore (Appendix E) and is activated to fluoresce in orange and/or yellow hues upon short and long wave UV light stimulation (Aubuchon V., http://www.vaughns-1-pagers.com/science/flourescent-mineral-colours.htm).

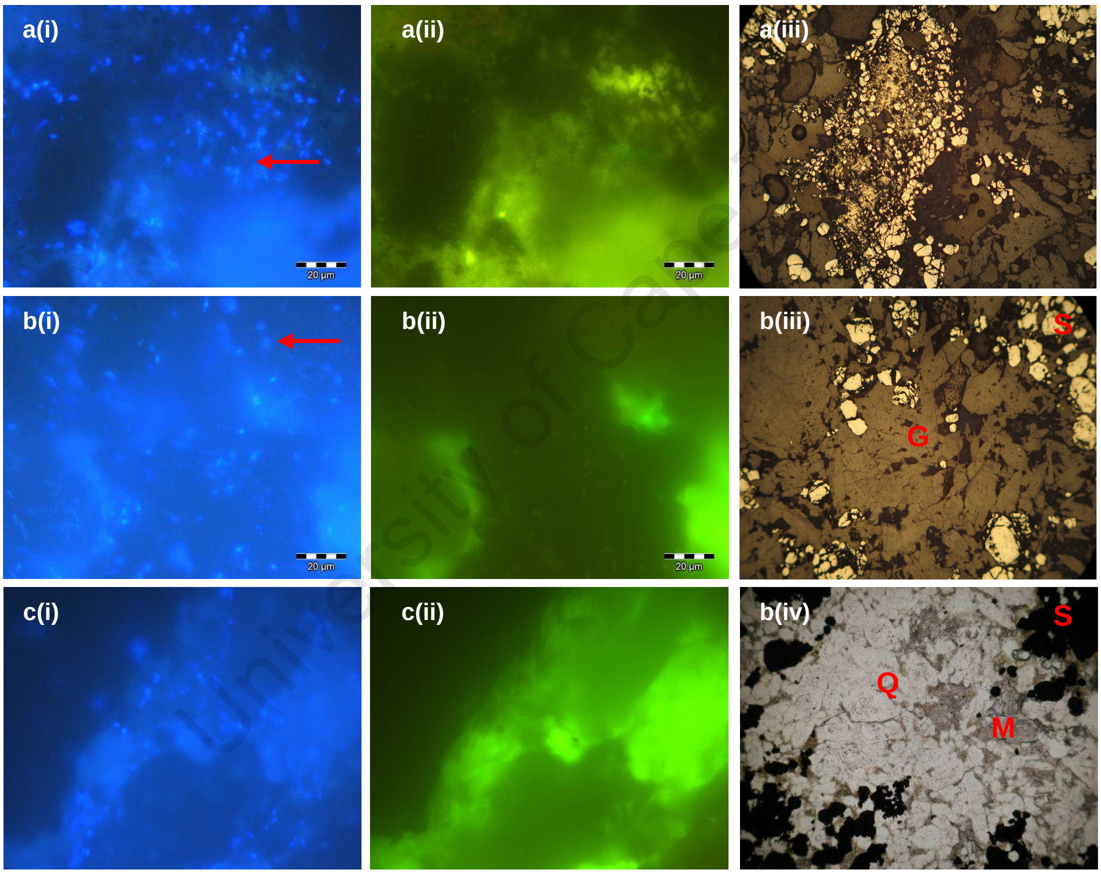

Figure 6.10: Biofilm reactor attachment of *A. ferrooxidans* and *L. ferriphilum* to the surface of a low-grade chalcopyrite containing mineral thin section, depicting **(i)** DAPI fluorescence, **(ii)** corresponding targeted FISH fluorescence, and **(iii)** the corresponding regions from mineralogical map under reflected light and **(iv)** transmitted light. Results observed for **(a)** Region 6b **(b)** Region 8b and **(c)** Region 8d.

Upon analysis of Figure 6.10, individual attached cells are discernable in Figure 6.10 (Ai) and (Bi) exhibited through positive fluorescence of the universal DAPI stain. Poor visual quality of cells which exhibited positive fluorescence of the *Leptospirillum* target (FISH) stain was evident. It was apparent that attachment did not occur exclusively to sulfide minerals in these regions as attached cells were evident in regions where the gangue (minerals quartz and muscovite) were present - evident upon comparison of the micrographs presented in Figure 6.10 (Ai), (Aii) and (Aiii), as well as (Bi), (Bii), (Biii) and (Biv). It is however important to note, that sulfide minerals are in close vicinity of these gangue minerals and the attached cells.

6.3.3 Biofilm reactor studies using low-grade chalcopyrite: Discussion

The biofilm reactor was successfully demonstrated as a novel, integrated *in situ* approach for the investigation of mineral-microbe interactions encompassing the use of low-grade chalcopyrite ores, mixed microbial consortia in an environment simulating heap attachment dynamics. Attachment and subsequent colonisation of mixed mesophilic cultures on the surface of low-grade chalcopyrite containing mineral ore thin section was demonstrated through the use of fluorescence microscopy. Individual attached cells were clearly visible on the surface of the thin section through the use of the universal counter-stain (DAPI). The use of the fluorescent labelled target (FISH) probe LF581 specific for *Leptospirillum* provided a sufficient means of identification and differentiation of attached microbial species in the two species consortium, as cells exhibiting positive fluorescence with LF581 were discernable. In some regions, FISH probe fluorescence was poor. This may have been due to background fluorescence caused by gangue minerals present or the absence of attached *L. ferriphilum* in those regions. Sterile conditions were maintained throughout the experiment thus the results of the attached colonies observed are indicative of the attachment of the microorganisms investigated.

Sulfide and gangue mineral identification and differentiation was accomplished through the use of ore microscopy and the examination of the intrinsic optical properties of the minerals concerned through the use of ore microscopy. A mineralogical map was constructed of the surface of the mineral thin section which was used to determine the spatial locale of attached microbial species. Microbial attachment was observed in regions on the surface of the low-grade chalcopyrite mineral section where sulfide minerals were predominant. Owing to the use of two separate microscopes (the Olympus for fluorescence microscopy, and the Zeiss for ore microscopy), with differing maximum magnification capabilities, it is recommended that tools for the mapping and comparison of mineralogical zone and attachment data be improved.

The observation of microbial attachment predominating in regions where sulfide minerals were abundant relative to gangue was consistent with the findings of Murr and Berry (1976) where the preferential attachment of bacteria to pyrite and chalcopyrite relative to siliceous phases in low-grade ore was demonstrated, as cited by Watling (2006). Findings were also consistent with the findings of the shake flask and column attachment studies presented and discussed in Chapters 4 and 5. Due to surface hydrophobicity and charge (Zobell 1943, van

Loosdrecht *et al*. 1987 and 1990, Rijnaart *et al.* 1993, Porro *et al.* 1997*,* Blake *et al.* 1994, Nagaoka *et al.* 1994, Sampson *et al.* 2000, Sharma *et al.* 2003), it was expected that cells will have a propensity to adhere to sulfide mineral phases in the low-grade chalcopyrite mineral ore thin sections. Attachment to regions containing gangue minerals (quartz and muscovite being dominant) was also observed at a low frequency.

Microbial attachment was dominant in regions where visible surface defects were prevalent, which was consistent with literature (Gehrke *et al.* 1998, Rojas-Chapana *et al.* 1998 and Harneit *et al.* 2005) as discussed in Section 6.2.2. The spatial distribution of the attached cells may have been influenced by fluid dynamics within the reactor environment, mineral surface effects (surface roughness and defects present) or affinity for the mineral through cell sensing. Further elucidation of these factors will form the subject of ongoing study.

6.4 CONCLUSIONS

A novel approach was developed with aim of allowing an adequate technique for visualising and investigating attachment to chalcopyrite ore sections in situ. Individual attached cells were visible using fluorescent microscopy techniques. Mono-layered biofilm formation was demonstrated under these experimental conditions and contact periods, which was consistent with literature reports. Attached cells were predominantly clustered, but not entirely limited to, regions where visible surface defects were prevalent. For experiments conducted using pure cultures, the density of the attached population was observed to increase as the contact period was extended from 72 hours to 96 hours. For experiments conducted using a mixed consortia, mixed micro-colonies of attached cells were evident. For low-grade chalcopyrite containing mineral ore thin sections, attached micro-colonies were observed predominantly in, but not limited to, regions where sulfide minerals were abundant. The establishment of firmly attached colonies was observed after 3.6 residence times. It is recommended that the FISH protocol be refined to mitigate the effects of background fluorescence by gangue.

To date no work has been reported in literature to assess biofilm development of a mixed microbial population on sulfide mineral surfaces. Further attachment studies have not been reported in flow through systems, representative of heap conditions, or in systems using low-grade chalcopyrite ores. The findings presented are novel and will be a topic of ongoing research.

Chapter 7: Conclusions

Industrial bioleaching operations may be carried out in stirred tank reactors or via the construction of heaps. This research focussed on heap bioleaching, a process of considerable potential for recovering copper from low grade ore bodies; however, it also has application to stirred tank bioleaching operations. The recovery of the copper via bioleaching occurs through microbially catalysed mineral dissolution. The attachment of microorganisms to the mineral surface is important when considering their involvement in bioleaching via the indirect contact mechanism (Crundwell 2003). Via this mechanism, attachment and subsequent production of EPS provides a reaction space with enhanced concentration of reactants required for more effective mineral dissolution. In a bio-heap context, successful attachment, colonisation and biofilm formation is critical in ensuring the proliferation of microbial life and improved heap leach efficiency. Considering gangue mineralogy, the use of mixed microbial consortia and continuous fluid flow become important factors for investigating attachment in a bio-heap context. In this study, the attachment of pure cultures to sulfide mineral concentrates and gangue containing copper sulfide ores was investigated in the agitated batch system using quartz as a control mineral. This was extended to study pure culture attachment using a continuous flow packed column reactor system and a novel *in situ* biofilm reactor system to simulate heap fluid flow dynamics. The latter was extended to mixed consortia.

Selective attachment of pure cultures of *A. ferrooxidans* to sulfide mineral ores over quartz was demonstrated using agitated batch systems. Rapid, selective attachment by *A. ferrooxidans* to sulfide minerals with preference given to sulfide minerals relative to low-grade chalcopyrite containing mineral ore and quartz was demonstrated, which supports the first hypothesis (Section 2.9). Greater than 90 % of the equilibrium attachment level was reached within 30 minutes of contacting microorganisms with the sulfide mineral concentrates. High levels of attachment were attained to sulfide minerals (92 % and 97 % attachment to pyrite and chalcopyrite respectively), with attachment to quartz and low grade chalcopyrite containing mineral ore reaching approximately half the maxima reached to sulfide minerals (52 % and 50 % attachment to low grade ore and quartz respectively) summarised in Table 4.1. These findings were in accordance with those reported in literature (Atkins *et al.* 1986, Escobar *et al.* 1996, Rodriguez *et al.* 2003, Harneit *et al.* 2005, Gonzalez *et al.* 1999) with some discrepancies in the maximum attachment reached reported by Ohmura *et al.* (1993) and Sampson *et al.* (2000). While this approach using agitated batch systems provided insight into the trends of microbial attachment to sulfide minerals and demonstrated consistency with previous studies, the quantitative results are not expected to represent attachment dynamics adequately in a heap environment. The findings with respect to the attachment of *A. ferrooxidans* to complex, low-grade sulfide-containing mineral ore are novel and will be the topic of further study.

The study was extended to continuous flow through systems in packed column reactors, described in Chapter 5, to simulate heap-like fluid dynamics. This approach has been demonstrated to be an effective means of investigating attachment under heap-like conditions. These observations and discussions were limited to bulk trends observed using pure cultures and mineral coated glass beads and focussed on the initial attachment stage of the colonisation process. This involves transport of the microorganism to the mineral surface followed by transient electrostatic and hydrophobic interactions between the surfaces before the onset of firm attachment. Initial adhesion interactions are thus governed by microbial and mineral surface properties (charge and hydrophobicity), with alterations in these properties influencing attachment behaviour.

For the investigation of the attachment of mesophilic microorganisms to all mineral systems in the packed column reactor, equilibrium attachment was achieved after 2 residence times (120 minutes). Preferential attachment to sulfide minerals over other minerals was observed under these experimental conditions which supports the first hypothesis (Section 2.9). For ore free cultured *A. ferrooxidans*, attachment efficiencies of 52 ± 4.3 %, 52 ± 1.5 %, 7 ± 4.7 % and 35 ± 0.8 % were observed to pyrite, chalcopyrite, gangue containing mineral ore and quartz respectively; with 55 ± 1.0 %, 27 ± 3.4 %, 26 ± 1.5 % and 51 ± 4.5 % observed to the respective minerals by ore free cultured *L. ferriphilum*. Findings of this study regarding selective attachment to sulfide minerals were in accordance with literature (Solari *et al.* 1992, Ohmura *et al.* 1993, Nagaoka *et al.* 1999, Gonzalez *et al.* 1999, Sampson *et al.* 2000, Rodriguez *et al.* 2003, Harneit *et al.* 2005) with discrepancies in maximum attachment efficiencies observed attributed to variations in experimental approach. Inoculum concentration may not be such an important factor governing the extent of initial adhesion interactions under these experimental conditions as the saturation cell number was never exceeded and the maximum coverage provided by the highest inoculum cell concentration was only 7 % of the total surface area available for attachment. *L. ferriphilum* and *A. ferrooxidans* exhibited similar trends of attachment and affinities for attachment, corroborated by statistical analysis of the variances across the results observed. Ore free cultured microorganisms exhibited a negative cell surface charge, in accordance with literature (Devasia *et al.* 1993, Ohmura *et al.* 1993, Blake *et al.* 1994 and Sharma *et al.* 2003). In all cases, it was found that the results were not due to a change in solution chemistry. The factors influencing initial adhesion interactions (surface electrokinetic potential and hydrophobicity) were the same for both mesophiles under these culture and experimental conditions.

Although the packed column approach simulated heap-like fluid dynamics, findings and discussions were limited to bulk trends observed using pure cultures and mineral coated glass beads and focussed on the initial attachment stage of the colonisation process. A novel *in situ* approach was developed, with the aim of developing an adequate technique for investigating and visualising attachment and subsequent colonisation to pure and low-grade chalcopyrite ore sections using pure and mixed microbial consortia. This is presented in Chapter 6. Results of this study provided insight into key questions around preferential microbe-mineral association, site specific attachment (i.e. attachment to surface defects) and spatial arrangement and organisation of attached microorganisms colonising the surface. They support the first hypothesis (Section 2.9).

Individual attached cells were visible using fluorescent microscopy techniques. Attached cells were predominantly clustered in regions where visible surface defects were prevalent. Low cell numbers were observed in the absence of surface defects. For experiments conducted using pure cultures, the density of the attached population was observed to increase as the contact period was extended, evident when comparing the 72 hour and 96 hour old biofilms. For experiments conducted using a mixed consortia, mixed micro-colonies of attached cells were evident. Mono-layered biofilm formation was demonstrated under these experimental conditions and contact periods, consistent with literature reports. For low grade chalcopyrite containing mineral ore thin sections, attached micro-colonies were observed predominantly in, but not limited to, regions where sulfide minerals were abundant. The establishment of firmly attached colonies was observed after 3.6 residence times. It is recommended that the FISH protocol be refined to mitigate the effects of background fluorescence by gangue. To date, no studies have been conducted to investigate the attachment of microorganisms to low grade chalcopyrite containing mineral ores, with few studies conducted using a mixed microbial consortium. These findings are novel and will be the topic of further study.

An appreciation for the factors influencing initial adhesion interactions i.e. transient, reversible electrostatic and hydrophobic interactions between the microbial and mineral surfaces (Zobell 1943, van Loosdrecht *et al.* 1990, Rijnaarts *et al.* 1993, Porro *et al.* 1997*,* Blake *et al.* 1994, Nagaoka *et al.* 1994, Sampson *et al.* 2000, Sharma *et al.* 2003) is fundamental to our understanding of the microbial adhesion mechanism. Initial interactions are governed by microbial and mineral surface properties (charge and hydrophobicity) and associated interface chemistry. Culture growth history has been correlated to changes in the surface properties of *A. ferrooxidans* (Porro *et al.* 1993, Rijnaarts *et al.* 1993, Blake *et al.* 1994, Gerhke *et al.* 1998, Kinzler *et al.* 2003, Sharma *et al.* 2003 and Harneit *et al.* 2005). This gave rise to key questions and the development of the second hypothesis (Section 2.9) regarding microbial growth history and the effect thereof on both microbial cell surface properties and subsequent attachment behaviour. In this study, the effects of varying culture conditions on cell surface charge (zeta-potential) and subsequent attachment ability were investigated using the packed column approach presented in Chapter 5 as well as the agitated batch system approach in Chapter 4. Microorganisms were cultured either on ferrous iron or adapted to a pyrite mineral concentrate.

Growth conditioning affected cell surface charge, which supports the second hypothesis (Section 2.9). Cultures adapted to pyrite mineral concentrate exhibited a positive cell surface charge and ore free cultured microorganisms exhibited a negative cell surface charge, consistent with literature reports (Devasia *et al.* 1993, Ohmura *et al.* 1993, Blake *et al.* 1994 and Sharma *et al.* 2003). Selective attachment to sulfide minerals over other minerals was also demonstrated using mineral-adapted cultures, which supports the first hypothesis (Section 2.9). The growth conditioning of mesophilic cultures using pyrite mineral adaptation and subsequent change in cell surface charge was correlated to an enhancement in the attachment efficiencies to sulfide mineral concentrates supporting the second hypothesis. Greater attachment efficiencies were achieved for mineral-adapted mesophilic cultures relative to ore free cultures, which is in accordance with literature (Gehrke *et al.* 1998). For mineral-adapted *A. ferrooxidans* attachment efficiencies of 74 ± 3.8 %, 63 ± 1.1 %, 25 ± 7.9 % and 23 ± 11.3 % were observed for the attachment to pyrite, chalcopyrite, gangue containing chalcopyrite mineral ore and quartz respectively; with 58 ± 2.6 %, 79 ± 2.6 %, 50 ± 4.1 % and 25 ± 7.2 % to each respective mineral system by mineral-adapted *L. ferriphilum*. For ore free cultured *A. ferrooxidans*, attachment efficiencies of 52 ± 4.3 %, 52 ± 1.5 %, 7 ± 4.7 % and 35 ± 0.8 % were observed to pyrite, chalcopyrite, gangue containing mineral ore and quartz respectively; with 55 ± 1.0 %, 27 ± 3.4 %, 26 ± 1.5 % and 51 ± 4.5 % observed to the respective minerals by ore free cultured *L. ferriphilum*. Enhanced attachment efficiencies observed for mineral-adapted cultures were attributed a net positive cell surface charge and increased cell surface hydrophobicity imparted through culturing on sulfide mineral concentrates was suggested to increase the extent of the attractive electrostatic interactions between microbial and mineral surfaces.

The highest overall attachment efficiencies were observed for mineral-adapted cultures to the pyrite mineral system. This was attributed to the fact that mineral adaptation was carried out using a pyrite mineral concentrate. Adaptation using pyrite effectively “primed” cells for attachment to sulfide minerals. It may be possible to enhance initial adhesion of microorganisms, make this attachment more predictable or selective for particular minerals by tailoring culture growth conditions. The impact of growth history on subsequent cell surface properties and attachment efficiencies is the topic of ongoing research. It is recommended that the pH range of the zeta potential study be increased and that the surface properties (both surface charge and hydrophobicity) be monitored throughout the mineral adaptation process and as a function of mineral used.

In this study, attachment trends of the thermophile *S. metallicus*, cultured under varying conditions, were investigated using the packed column approach at ambient temperature, presented in Chapter 5. No explicit attachment trend was evident. Both ore free and chalcopyrite mineral-adapted *S. metallicus* cultures washed out after 1.5 residence times (90 minutes) with the exception of mineral-adapted *S.metallicus* contacted with pyrite where washout was only observed after two residence times (120 minutes). The wash out occurred more rapidly for ore free cultures as mineral-adapted cultures exhibited high initial cell retention. This may be indicative of enhanced or selective association of mineral-adapted cultures to the mineral surface relative to ore free cultures. Highest overall attachment (28 ± 9.9 %) was observed for chalcopyrite mineral-adapted cultures to the pyrite mineral system, suggesting that mineral-adapted *S. metallicus* exhibit an enhanced affinity for attachment to pyrite, and attach preferentially to pyrite over chalcopyrite. Growth of cultures on sulfide mineral enhanced the extent of subsequent attachment to sulfide minerals. While these data are relevant to the start up phase of a heap bioleach where temperatures are ambient, they are expected to underestimate the maximum levels of attachment attainable for *S. metallicus* due to sub-optimal thermal conditions influencing microbial activity. The standard deviations of the results reported for *S. metallicus* were high. It is recommended that the experiments for the study of the attachment of *S. metallicus* be extended to provide refined data under both ambient and optimal temperature conditions for *S. metallicus*. Inoculum should be optimised to minimise loss of microbial activity (harvesting of cells should not be conducted at 4 ˚ C).

The attachment of microorganisms plays an important role in the bioleach process explained via the “indirect contact mechanism” (Crundwell 2003). Furthermore, successful attachment, colonisation and biofilm formation warrants the proliferation of microbial life and improved heap leach efficiency. Industrial bio-heap operations are carried out using complex, low-grade (gangue containing) copper ores. An appreciation of mineralogy, microorganisms, how microorganisms associate and interact with each other, as well as understanding how this dynamic changes as the leach progresses is fundamental to overall understanding of the bioleach process, the role of microbes, and more specifically the role of microbial attachment therein. The findings of this study provide valuable incremental knowledge to bio-heap microbial attachment and colonisation dynamics through simulation of bio-heap conditions by considering mixed microbial consortia, fluid flow, gangue mineralogy and integrated *in situ* investigations. The latter two considerations (gangue mineralogy and *in situ* studies) provide novel contributions to knowledge in a microbial-attachment and bioleach context. This increases the understanding of the role and importance of attachment in the bioleach process as explained via the “indirect contact mechanism”, and contributes to the mitigation of challenges currently faced by industry (Chapter 1). The findings of this study provide insight into: the mechanism of microbial adhesion to mineral surfaces, bulk trends in microbial attachment, spatial attachment, site specific and preferential microbe-mineral association, as well as the establishment and spatial organisation of mixed microbial biofilms under conditions representative of a bio-heap. Furthermore, attention is drawn to the role of initial adhesion interactions in the attachment-colonisation process, governed by microbial and mineral surface properties. In appreciation of initial adhesion interactions and the knowledge gained in this study, the potential for the tailoring culture growth conditions to prime attachment to specific minerals or make attachment more predictable is highlighted.

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Appendix A: Media and buffer composition

Growth media used for ore free cultured microorganisms:

***A. ferrooxidans* growth medium**	
Chemical	**Mass (g)**
$(NH_4)_2SO_4$	2
KCl	0.1
$MgSO_4.7H_2O$	0.5
K_2HPO_4	0.5
$FeSO_4.7H_2O$	8

The salts were dissolved in 950 ml distilled water and the pH adjusted to 2.0 with H_2SO_4. The solution was then autoclaved. The ferrous iron was dissolved in 50 ml distilled water and purified using a 0.45 µm filter and then added to the autoclaved salt solution.

***L. ferriphilum* growth medium**	
Chemical	**Mass (g)**
$(NH_4)_2SO_4$	0.132
K_2HPO_4	0.027
$MgCl_2.6H_2O$	0.053
$CaCl_2.2H_2O$	0.147
$FeSO_4.7H_2O$	20
Trace element solution	
$MnCl_2.2H_2O$	0.062
$ZnCl_2$	0.068
$CoCl_2.6H_2O$	0.064
H_3BO_3	0.031
Na_2MoO_4	0.01
$CuCl_2.2H_2O$	0.067

The salts were dissolved in 950 ml distilled water and the pH adjusted to 1.8 with H_2SO_4 and autoclaved. The ferrous iron was dissolved in 50 ml distilled water and purified using a 0.45 µm filter and then added to the autoclaved salt solution. The trace element solution was dissolved in 1 L distilled water and the pH adjusted to 1.8. A volume of 1 ml of trace element solution was added to the autoclaved solution.

***S. metallicus* growth medium**	
Chemical	**Mass (g)**
$(NH_4)_2SO_4$	1.30
K_2HPO_4	0.28
$MgSO_4.7H_2O$	0.25
$CaCl_2.2H_2O$	0.07
Yeast extract	1.00
Elemental Sulphur	0.05 g

The salts and yeast extract was dissolved in 1 L distilled water and the pH adjusted to 2.0 using H_2SO_4. The solution was then autoclaved. Subsequently, 0.05 gL^{-1} elemental sulphur was added. Finally, a volume of 1 ml of the trace element solution was added.

9 K Basal salts medium:

Chemical	Mass (g)
$(NH_4)_2SO_4$	3
KCl	0.1
$MgSO_4.7H_2O$	0.5
K_2HPO_4	0.5
$Ca(NO_3)_2.4H_2O$	1.45

The media was dissolved in 1 L distilled water, the pH of the medium adjusted to the desired value using concentrated H_2SO_4 and then autoclaved for 20 minutes at 120˚ C.

1 x Phosphate Buffered Saline (PBS):

Chemical	Mass (g)
NaCl	8
KCl	0.2
Na_2HPO_4	1.44
KH_2PO_4	0.24

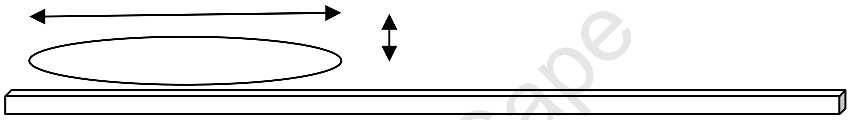
The media was dissolved in 0.8 L distilled water. The pH of the medium adjusted to pH 7.0 using hydrochloric acid (HCl). The volume was then made up to 1 L with distilled water and then autoclaved for 20 minutes at 120˚ C.

Appendix B: Quantification of maximum attachment per unit surface area

Quantification of the maximum attachment per available surface area provided information on whether or not the column had been saturated by the inoculum. Saturation of the surface available for attachment by the inoculum may influence the final attachment efficiencies observed. Thus, these calculations allowed the impact of inoculum concentration on the attachment to be analysed and allowed more adequate comparison of extent of attachment across experiments.

Calculation of the maximum attachment per unit surface area was carried out as follows and by making the following assumptions:

- Repulsive forces between cells and minerals were ignored
- Attachment was monolayered with cells orientated such that the major axis was parallel to the surface of the mineral coated bead as depicted below



- Dimensions of the mesophilic microorganisms used: 1.5 x 0.5 µm

  Surface area of microbial cell is therefore: 0.75 µm^2
  Dimensions used were based on the findings of Ohmura *et al.* (1993) for *A. ferrooxidans*.

- For column experiments the surface area available for attachment was calculated by quantifying the total surface area of the spherical glass beads housed in the reactor

  Average diameter of spherical glass bead: 6 x 10^{-3} m
  Number of beads used: 300
  Total surface area made available by spherical, mineral coated, glass beads

  $$= 300 * \left(4 * \pi * r^2\right)$$
  $$= 3.4x10^{-2}\ m^2$$

- The maximum number of *A. ferrooxidans* cells that could be accommodated by the available surface area in the packed column reactor was calculated. This number will be referred to as the saturation cell number.

  Saturation cell number calculation:

$$= \frac{Total\ surface\ area\ made\ available\ for\ attachment\ by\ coated\ glass\ beads\ [m^2]}{Surface\ area\ per\ microbial\ cell\left[\frac{m^2}{cell}\right]}$$

$$= \frac{3.4x10^{-2}}{7.5x10^{-13}}$$

$$= 4.5x10^{10}\ cells$$

- The cumulative proportion of cells retained in the reactor vessel was quantified by subtracting the cell concentration of eluted samples from the total inoculum cell number and can be given by the following equation:

  Cells retained in the reactor $=(Co - C)$

  Where:
  Co = inoculum cell number
  C = cell number in eluted samples (cumulative)

  Attachment efficiency per unit surface area was performed by dividing the cumulative proportion of cells retained in the reactor by the total surface area available for attachment.

$$= \frac{Cumulative\ number\ of\ cells\ retained\ in\ the\ reactor\ [cells]}{Total\ surface\ area\ made\ available\ for\ attachment\ by\ coated\ glass\ beads\ [m^2]}$$

Appendix C: Column residence time calculation, study solution chemistry results and raw data

C1: Residence time calculation

The residence time of a column packed with chalcopyrite mineral coated beads was conducted using step introduction of a pH tracer (Fogler 2006). The experiment was conducted in duplicate. The pH tracers employed were; 1 x PBS (pH 7.05) and H_2SO_4 (pH2.05). The reactor was operated as a continuous flow through system as a constant flow rate of 1ml min^{-1}.

The H_2SO_4 tracer was introduced into column and the pH monitored until a pH of 2.05 was measured in the exiting solution. Once this pH was reached and maintained, the PBS tracer was introduced and pH monitored until a pH of 7.07 was measured in the exiting solution. The H_2SO_4 tracer was then introduced again and the pH monitored until a pH of 2.05 was measured in the exiting solution.

The residence time distribution function was determined. The function describes how much time particular fluid elements have spent in the reactor in a quantitative manner (Fogler 2006). The function is shown below.

$$E(t) = \left(\frac{C(t_1) - C(t_0)}{C_0(t_1 - t_0)} \right) \qquad \text{(Eqn C1)}$$

- C, refers to the concentration of fluid elements being monitored, which in this case would be the H+ ion concentration (pH = - log H+).
- C_o refers to the initial H+ concentration

The residence time distribution function was plotted as a function of time. The area under the graph allows indication of the time spent in the reactor by fluid elements. Residence time was calculated through determination of the area under the graph using the trapezium method.

The residence time was calculated to be 52 minutes and then 56 minutes.

The void volume was determined to be 55 ml. This is in agreement with the residence time calculation.

Residence time distribution raw data follows.

Table C1: Residence time distribution raw data (used to determine a residence time of 56 minutes)

Time (minutes)	pH	Time (minutes)	pH	Time (minutes)	pH
0	3.06	52	7.05	43	6.39
1	3.07	53	7.07	44	6.34
2	3.1	54	7.08	45	6.3
3	3.14	55	7.09	46	6.28
4	3.2	56	7.1	47	6.24
5	3.26	57	7.14	48	6.19
6	3.3	58	7.15	49	6.12
7	3.33	59	7.17	50	6.1
8	3.38	60	7.18	51	6.06
9	3.41	0	7.18	52	6.04
10	3.45	1	7.15	53	6.02
11	3.47	2	7.11	54	5.99
12	3.49	3	7.1	55	5.96
13	3.5	4	7.09	56	5.93
14	3.51	5	7.08	57	5.91
15	3.53	6	7.07	58	5.86
16	3.54	7	7.06	59	5.8
17	3.55	8	7.05	60	5.72
18	3.56	9	7.04	61	5.64
19	3.57	10	7.03	62	5.37
20	3.58	11	7.02	63	5.13
21	3.59	12	7.01	64	4.8
22	3.6	13	6.99	65	4.55
23	3.61	14	6.98	66	4.37
24	3.62	15	6.97	67	4.3
25	3.65	16	6.96	68	4.21
26	3.67	17	6.95	69	4.07
27	3.71	18	6.94	70	3.91
28	3.73	19	6.93	71	3.89
29	3.74	20	6.92	72	3.77
30	3.75	21	6.91	73	3.65
31	3.77	22	6.89	74	3.56
32	3.84	23	6.87	75	3.5
33	3.93	24	6.86	76	3.44
34	4	25	6.84	77	3.4
35	4.08	26	6.82	78	3.38
36	4.14	27	6.81	79	3.37
37	4.2	28	6.79	80	3.36
38	4.25	29	6.77	81	3.35
39	4.34	30	6.75	82	3.34
40	4.88	31	6.74	83	3.33
41	5.74	32	6.71	84	3.32
42	6.13	33	6.69	85	3.31
43	6.35	34	6.64	86	3.3
44	6.51	35	6.62	87	3.29
45	6.65	36	6.59	88	3.28
46	6.74	37	6.57	89	3.27
47	6.84	38	6.56	90	3.26
48	6.89	39	6.52		
49	6.94	40	6.5		
50	6.98	41	6.46		
51	7.01	42	6.43		

C2: Solution chemistry results for Column attachment study

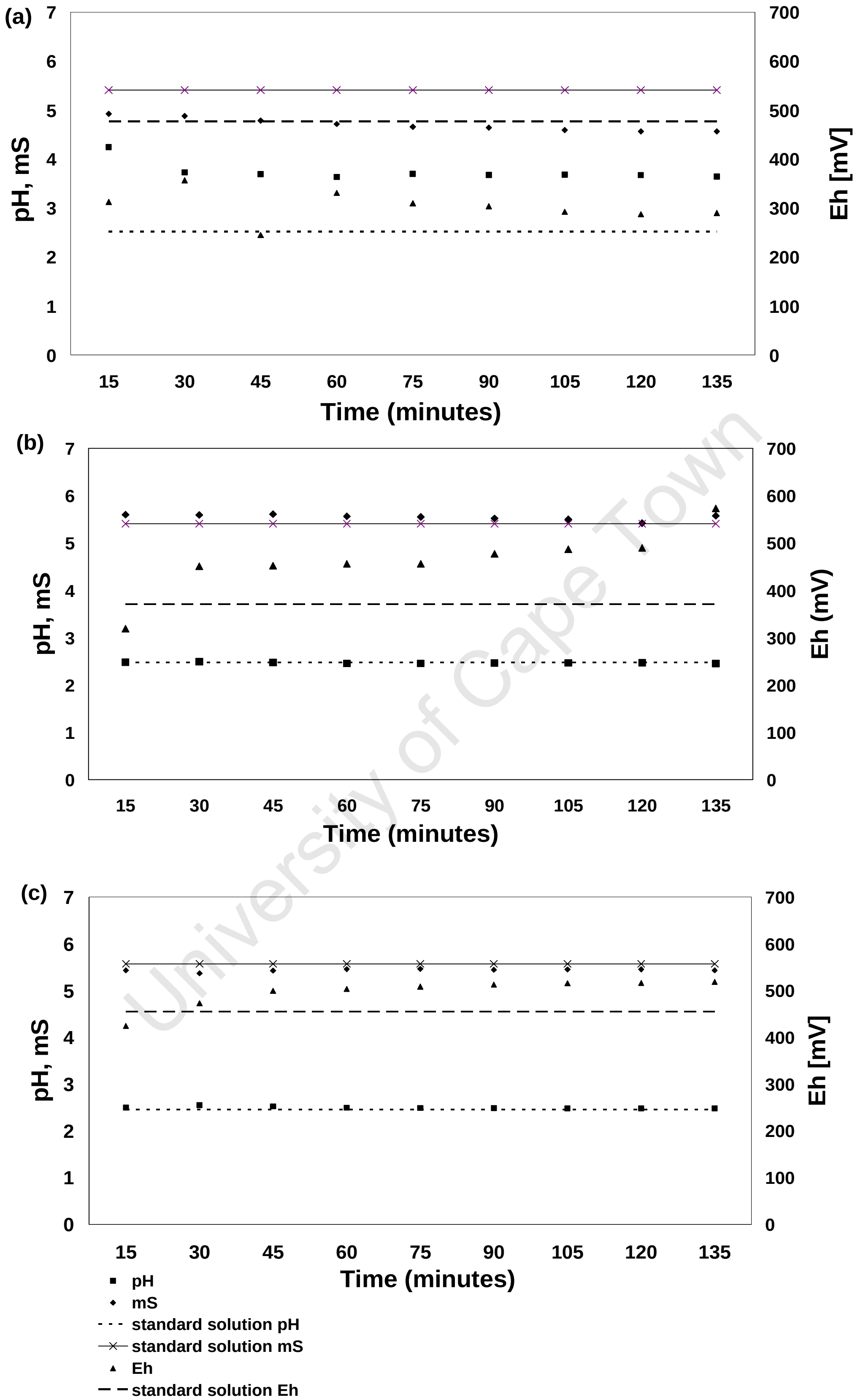


Figure C.1: Solution chemistry of mineral adapted *L. ferriphilum* to **(a)** pyrite, **(b)** quartz and **(c)** low grade chalcopyrite containing mineral ore.

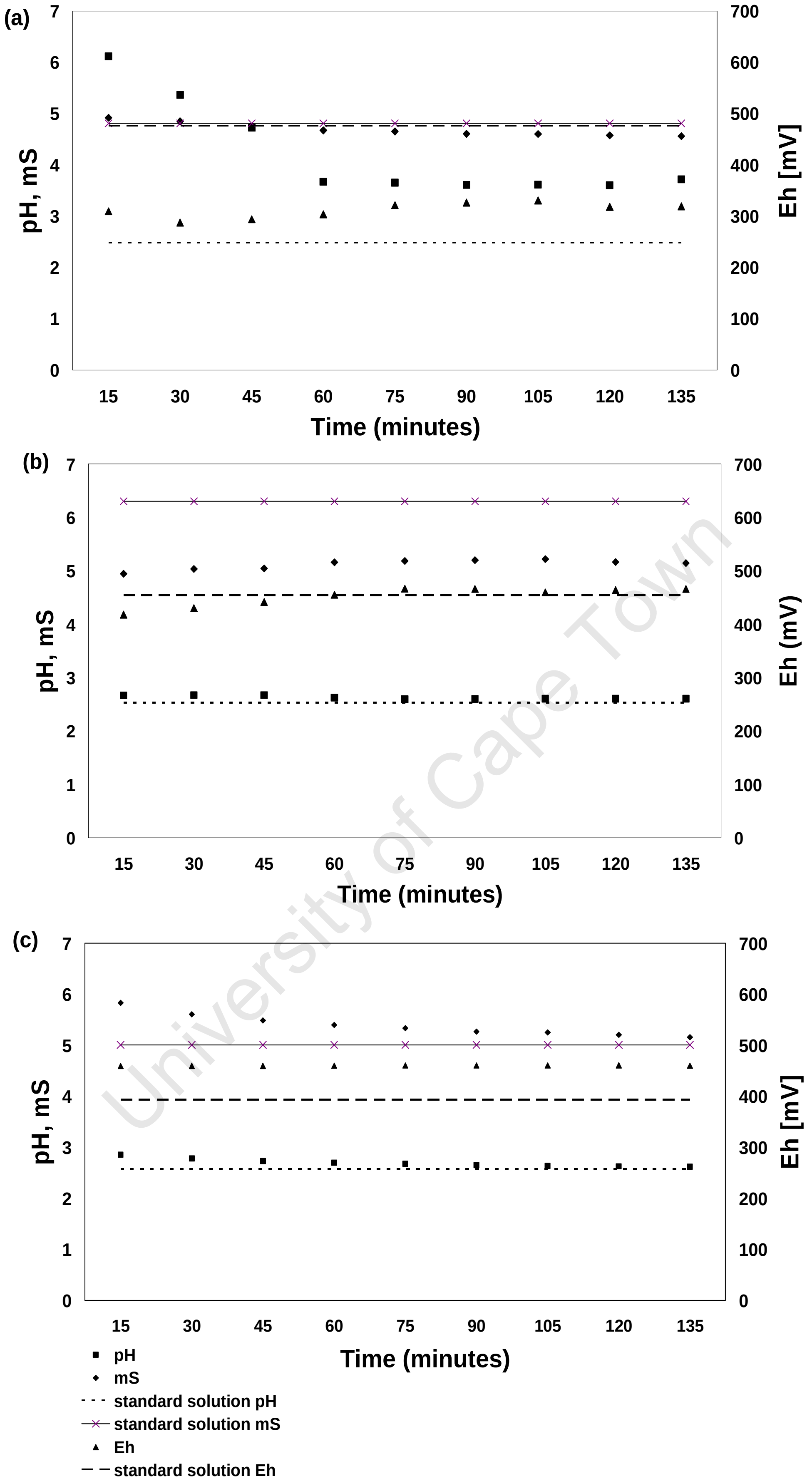


Figure C.2: Solution chemistry of mineral adapted *A. ferrooxidans* to **(a)** pyrite, **(b)** quartz and **(c)** low grade chalcopyrite containing mineral ore.

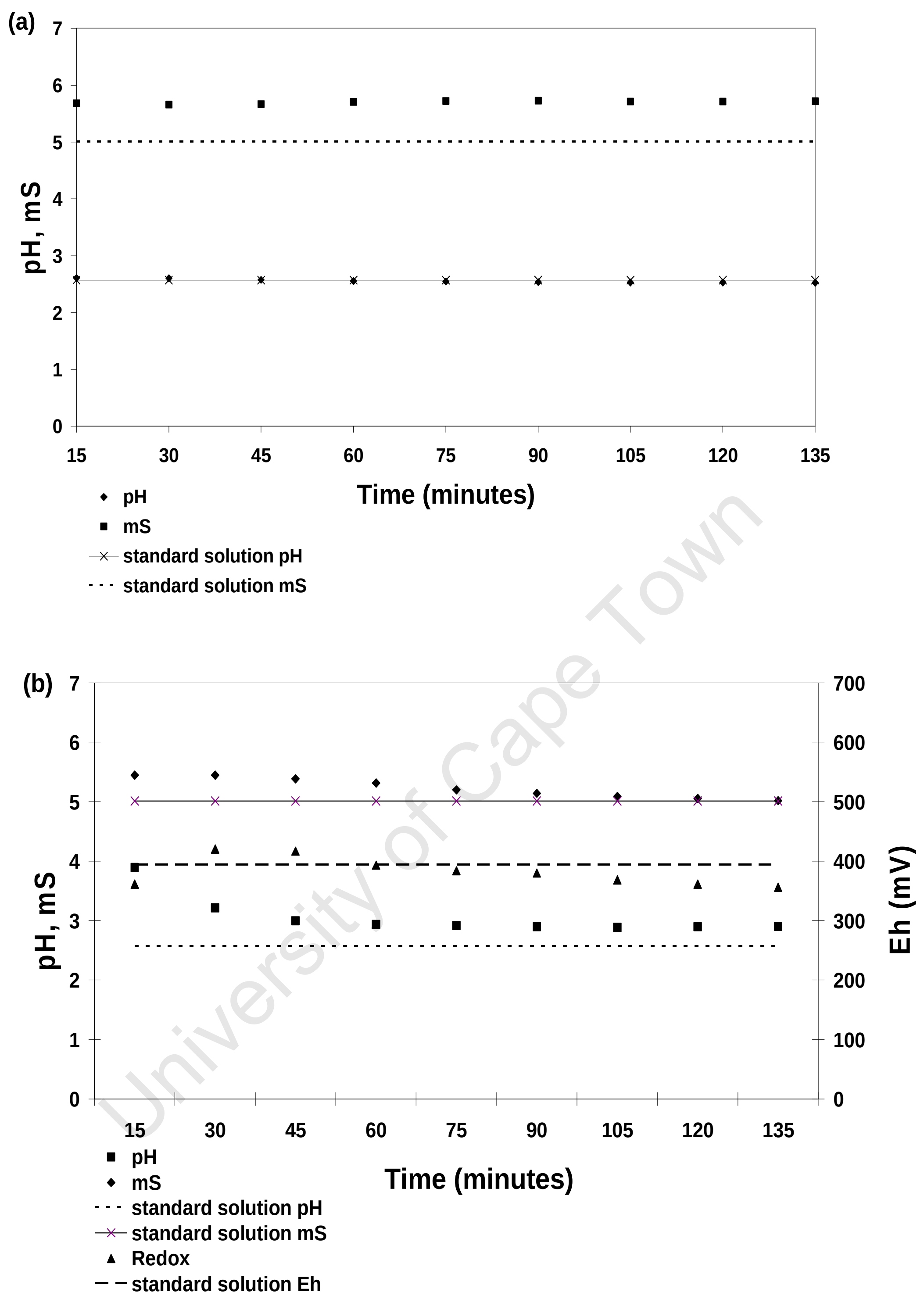


Figure C.3: Solution chemistry of ore free cultured *S. metallicus* to **(a)** chalcopyrite and **(b)** pyrite mineral concentrates.

Appendix D: Analysis of variance (ANOVA) of column data using

ANOVA or analysis of variance is "a statistical method for making simultaneous comparisons between two or more means; a statistical method that yields values that can be tested to determine whether a significant relation exists between variables" (George A. Miller, http://wordnetweb.princeton.edu/perl/webwn?s=anova).

ANOVA (Two-Factor with Replication is useful) when data can be classified along two different dimensions. Such is the case in this study as attachment of microorganisms was investigated. The extent of which may have been affected by; the microbial species used as these were different (*A. ferrooxidans* and *L. ferriphilum*), and the substrata used as four mineral substrata were used (pyrite, chalcopyrite, low grade chalcopyrite and quartz). For each battery of tests (microorganism-mineral) there are an equal number of observations of attachment (which was conducted in triplicate). Using this ANOVA tool it is possible to test:

1. Whether the attachment of the two microbial species are drawn from the same underlying population (differences due to mineral system ignored)
2. Whether the attachment of microorganisms to different mineral systems are drawn from the same underlying population (with differences in microbial species ignored)

Thus, the use of ANOVA (Two-factor with replication) was two fold. Firstly ANOVA was performed to establish whether or not any similarities existed between the extent of attachment between *A. ferrooxidans* and *L. ferriphilum*. If the attachments of the two microbial species are drawn from the same underlying population then the results are comparable and similarities existed between the extents of attachment of these microorganisms to particular minerals. There would be statistically significant evidence to suggest that the behaviour of these two mesophilic microorganisms in their attachment to a particular mineral was similar.

Null hypothesis H_0: the two microbial species are drawn from the same underlying population and there is no significant difference between the extents of attachment, and thus behaviour observed between *A. ferrooxidans* and *L. ferriphilum*.

Alternate hypothesis H_A: There is a statistically significant difference between the extents of attachment and thus behaviour observed between *A. ferrooxidans* and *L. ferriphilum*.

Results ($F < F_{crit}$)

$F_{crit} = 3.89$

$F = 2.108$

- Reject Null in favour of alternate hypothesis if F > Fcrit.
- If F < Fcrit then there is no statistically significant difference. between the extent of the attachment of the two mesophiles to that mineral and thus no difference in there attachment behaviour.

Since F (2.108) < F*crit* (3.89), with a 95 % confidence and a p value of 0.05, we accept the null hypothesis that there is no statistically significant difference between the extents of attachment observed between *A. ferrooxidans* and *L. ferriphilum* system. Thus, these two mesophiles are observed to behave in a similar fashion and there is statistically significant evidence to suggest that the extent of attachment of these two microorganisms to the various minerals is similar

The second use of ANOVA was to determine whether the attachment of microorganisms to different mineral systems is drawn from the same underlying population. If they are so then the results are comparable. If no similarities existed between the adherence of these microorganisms to the various mineral systems then, the attachment observed could not be attributed to a chance event and was a function of a particular mineral system.

<u>Minerals:</u>

Null hypothesis H_0: There is no significant difference in the extent of attachment attained between the various mineral systems (pyrite, chalcopyrite, low grade chalcopyrite containing mineral ore and quartz).

Alternate hypothesis H_A: There is a significant difference in the extent of attachment attained between the various mineral systems (pyrite, chalcopyrite, low grade chalcopyrite containing mineral ore and quartz).

Results: (F> F*crit*)
F*crit* = 1.839
F = 6.124

For samples: F (6.124) > F*crit* (1.839), with a 95 % confidence and a p value of 0.05, we reject the null hypothesis in favour of the alternate. There is a statistically significant difference between the extents of attachment observed between various mineral systems. The differences can not only be explained by chance. There is thus statistically significant evidence to suggest that the extents of attachment observed to each mineral system is a function of the mineral in question. (else variances of attachment efficiencies across all mineral types would be similar i.e. there would be no statistical difference).

Table D1: ANOVA (Two-Factor With Replication) Test Results

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Mineral systems	3.067	11	0.279	6.124	1E-08	1.838792
Microorganisms	0.096	1	0.096	2.108	0.1481	3.890349
Interaction	0.6304	11	0.057	1.259	0.2513	1.838792
Within	8.742	192	0.046			
Total	12.535	215				

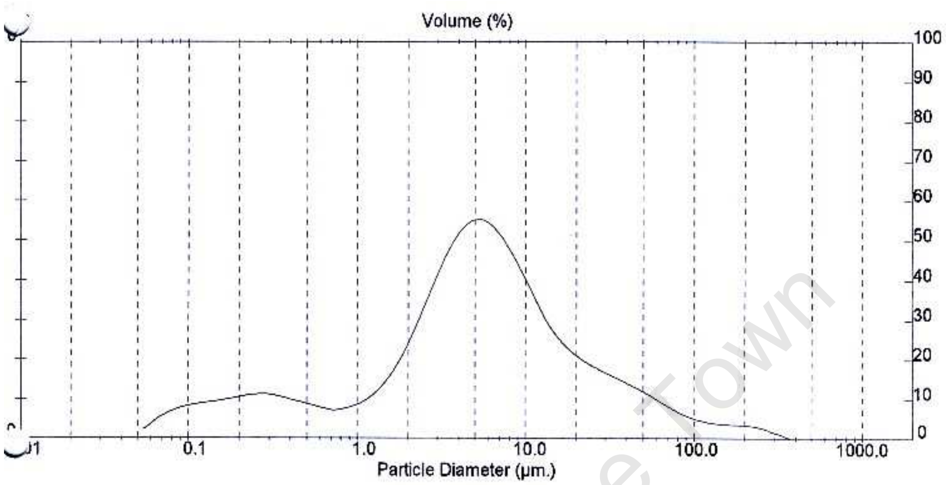
Table D2: ANOVA Test Raw data

Mineral system	**Microorganisms**	
	***A. ferrooxidans* (MA)**	***L. ferriphilum* (MA)**
Pyrite	99.7%	97.1%
	92.2%	92.3%
	84.0%	88.7%
	79.3%	85.8%
	77.0%	84.6%
	74.7%	83.6%
	72.7%	82.7%
	71.2%	81.8%
	70.0%	81.8%
	99.1%	98.1%
	90.4%	91.4%
	86.0%	87.8%
	84.5%	85.6%
	83.2%	83.5%
	81.4%	81.9%
	79.8%	81.0%
	78.6%	80.4%
	77.7%	80.0%
	99.8%	98.0%
	93.2%	89.1%
	84.0%	85.4%
	82.0%	82.3%
	79.4%	80.0%
	77.6%	78.5%
	76.0%	77.6%
	74.5%	76.9%
	73.4%	76.7%
Chalcopyrite	100.0%	96.8%
	80.1%	76.4%
	74.3%	71.7%
	70.4%	68.7%
	67.9%	67.0%
	66.0%	65.1%
	65.1%	63.2%
	64.5%	61.7%
	63.9%	60.3%
	100.0%	96.0%
	80.2%	73.7%
	72.8%	68.0%
	69.0%	64.7%
	66.7%	62.0%
	65.1%	60.2%
	64.5%	58.6%
	63.9%	57.0%
	63.2%	55.5%
	100.0%	95.5%
	77.9%	76.3%
	71.9%	71.9%
	68.1%	68.7%
	65.7%	66.3%

	64.2%	64.4%
	63.0%	62.5%
	62.1%	61.2%
	61.7%	59.6%
Low grade chalcopyrite	99.7%	100.0%
	74.7%	99.8%
	57.2%	85.6%
	47.9%	69.4%
	39.7%	59.1%
	34.1%	54.1%
	31.1%	50.7%
	29.2%	48.6%
	27.5%	47.7%
	99.7%	99.8%
	76.1%	98.0%
	60.4%	79.8%
	50.9%	68.0%
	43.1%	62.5%
	38.4%	59.5%
	34.9%	57.1%
	32.7%	55.7%
	31.1%	55.2%
	99.4%	99.8%
	69.6%	97.3%
	50.6%	78.3%
	39.9%	64.7%
	30.7%	58.3%
	24.4%	53.1%
	19.9%	50.2%
	17.1%	49.2%
	16.0%	48.5%
Quartz	100.0%	99.8%
	99.8%	98.5%
	93.5%	81.3%
	65.1%	55.8%
	38.6%	36.8%
	22.2%	34.0%
	16.4%	32.5%
	14.4%	31.3%
	13.5%	29.5%
	100.0%	100.0%
	100.0%	97.1%
	96.4%	79.2%
	70.7%	52.6%
	47.7%	37.1%
	42.1%	32.5%
	37.4%	29.8%
	35.7%	28.9%
	35.3%	27.9%
	100.0%	99.8%
	100.0%	97.3%
	95.0%	70.5%
	64.6%	38.3%
	33.3%	28.8%
	24.1%	22.0%
	20.9%	19.8%
	19.8%	18.3%
	19.0%	16.3%

Appendix E: Mineralogical information of substrata used

Below, the results of the size analysis showing the particle size distributions of the chalcopyrite (Andina mine) and pyrite (Agnes mine) mineral concentrate samples are presented.



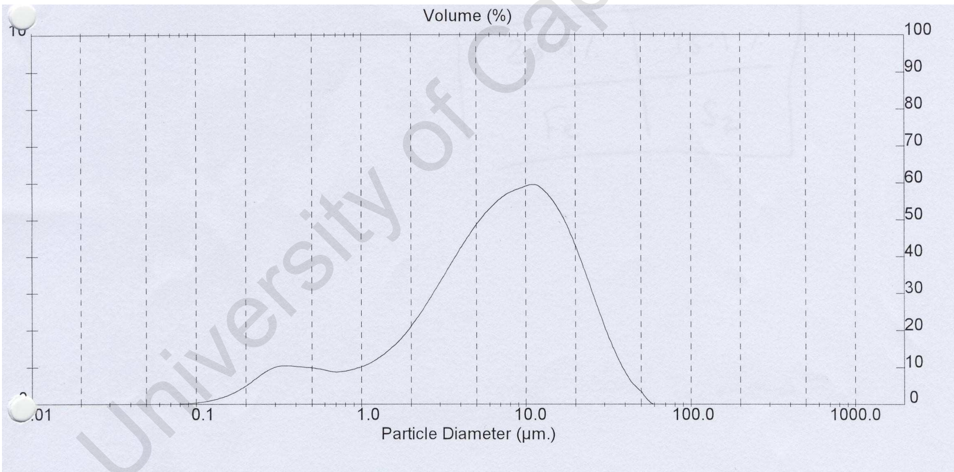


Figure E1: Chalcopyrite mineral concentrate (Andina mine) size analysis results (top) and Pyrite mineral concentrate (Agnes mine) size analysis results (bottom).

Table E1: Summary of the particle size distribution information for the pyrite and chalcopyrite mineral samples used.

Mineral Sample	d10 (μm)	d50 (μm)	d90 (μm)
Pyrite	0.89	7.08	21.78
Chalcopyrite	0.31	5.25	33.10

Table E2: The mineralogical composition of low chalcopyrite containing mineral ore (Escondida, CH32-Type B) used in this study is presented below, with predominating sulfide minerals highlighted in gold, and predominating gangue minerals highlighted in green.

Minerals	CH32-Type B
Chalcocite	0.2
Chalcopyrite	0.5
Covellite	0.3
Bornite	0.1
Atacamite	0.0
Brochantite	0.0
Chrysocolla	0.0
Cuprite	0.0
Native_Copper	0.0
Tenorite	0.0
Turquoise	0.0
Enargite	0.1
Pyrite	4.0
Sphalerite	0.0
Molybdenite	0.0
Galena	0.0
Ilmenite	0.0
Monazite	0.0
Rutile	0.2
Thorite	0.0
Zircon	0.0
Muscovite	28.6
Biotite	0.5
Chlorite	0.7
Kaolinite	7.4
Calcite	0.0
Goethite	0.1
Hematite	0.2
Magnetite	0.0
Albite	5.7
Na_orthoclase	2.9
Plagioclase	1.7
Alunite	0.5
Jarosite	0.2
Gypsum	0.0
Barite	0.0
Svanbergite	0.6
Apatite	0.1
Hornblende	0.2
Iron	0.0
Chromite	0.0
Corundum	0.0
Aluminium_Phosphate	0.0
Quartz	44.8

Michel-Levy Interference Colour Chart

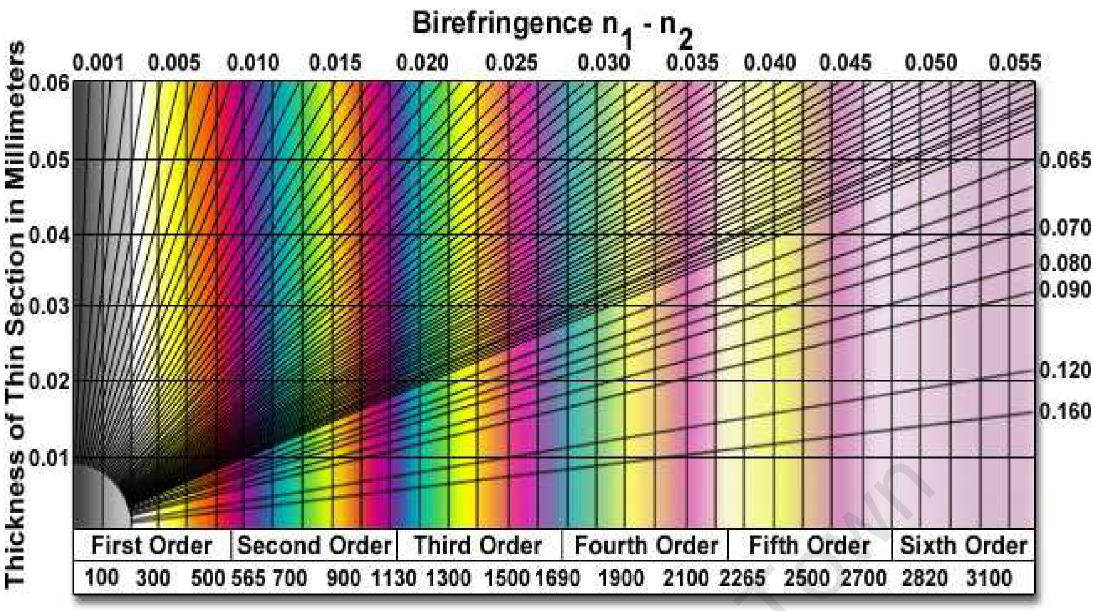


Table E.3: Summary of the predominating sulphide and gangue minerals present (wt %) in th Escondida CH-32-Type B ore (courtesy of BHP Billiton), as well as a summary of the optical properties used for mineral identification under reflected and transmitted light using ore microscopy (Rodgers 1937, Anthony *et al.* 1990 and Deer *et al.* 1992).

Mineral	Chemical composition	wt%	Characteristic optical properties		
			Reflected Light		Transmitted Light
Major sulphide minerals					
Pyrite	FeS_2	4.0	Cream to pale yellow	Black	
Chalcopyrite	$CuFeS_2$	0.5	Brassy yellow	Black	
Covellite	CuS	0.3	Green intergrowth on chalcopyrite surface	Black	
Chalcocite	Cu_2S	0.2	Pale grey	Black	
Major gangue minerals					PPL XPL
Quartz	SiO_2	44.8	Dull brown grey (does not reflect light)	Colourless to milky in thin sections	Isotropic Exhibits weak birefringence (0.009) with light to darker greys and white interference colours
Muscovite	$KAl2(Si_3Al)O_{10}(OH;F)_2$	28.6	Dull brown grey (does not reflect light)	Colourless in thin section, may appear tinted in hues of grey or brown	Anisotropic Exhibits high birefringence (0.035 - 0.042) with interference colours ranging from the violet through to red spectrum

Table E.4: The major sulfide and gangue minerals present in the Kennecott ore sample, and the associated characteristic optical properties used for their identification is presented.

Sulfide minerals

Mineral	Mineral formulae	Characteristic optical properties	
		Reflected light	Transmitted light
Chalcopyrite	$CuFeS_2$	Brassy yellow	Black
Pyrite	FeS_2	Pale yellow	Black
Molybdenite	MoS_2	Light grey	Black

Gangue minerals

Mineral	Mineral formulae	Reflected light	Transmitted light	
			PPL	XPL
Quartz	SiO_2	Dull brown to grey (does not reflect light)	Colourless to milky or white in thin sections	Exhibits weak birefringence (0.009) Isotropic Light to darker greys and white interference colours
Plagioclase	$(Na,Ca)\ Al(Al,Si)\ Si_2O_8$	Dull brown to grey (does not reflect light)	White to light-grey and colourless, may display lamellar twinning	Exhibits weak birefringence (0.007- 0.013)
Orthoclase	$KAlSi_2O_8$	Dull brown to grey (does not reflect light)	Colours may vary from white to colourless, cream to tan, may display cross hashed twinning May exhibit cross hash twinning	Exhibits weak birefringence (0.006-0.010) Extinction angle occurs between 5 to 19º
Biotite	$K(Mg,Fe,Al)_3\ AlSi_3O_{10}(OH)_8$	Dull brown to grey (does not reflect light)	Black or dark brown	Strong birefringence (0.028-0.08) Exhibits pleochroism
Chlorite	$(Cl,\ Mg,Fe,Al)_6\ AlSi_3O_{10}(OH)_2$	Dull brown to grey (does not reflect light)	Green of various tints, varying from almost white to almost black.	Exhibits weak to moderate birefringence (0.007-0.015) With yellow to green to very dark interference colours.
Calcite	$CaCO_3$	Dull brown to grey (does not reflect light)	Clear or white, to tan or grey. May display lamellar twinning.	May be tinted many colours and is anisotropic. Displays rainbow pleochroism. Exhibits strong birefringence 0.172-(0.190).
Amphibole (Hornblend)	$mCa(Mg,Fe)_3\ (SiO_3)_4 + n(Al,Fe)(F,OH)\ SiO_3$	Dull brown to grey (does not reflect light)	Green to brown	Exhibits birefringence (0.022 – 0.027) With moderate to strong, white and/or yellow interference colours. Extinction at 10 to 20˚

Appendix F: Biofilm reactor residence time calculation and flow rate rationale

biofilm specs		
diameter reactor (mm)	100.00	100.00
flow rate (µl min^{-1})	60.00	300.00
glass slide:		
length (mm)	75.00	75.00
width (mm)	26.00	26.00
film thickness (mm)	0.50	0.50
volume fluid on glass slide (mm^3)	975.00	975.00
volume fluid on glass slide (ml)	0.98	0.98
volume fluid on glass slide (µl)	975.00	975.00
volume fluid passing through the reactor (µl)	20000.00	20000.00
surface area film (height film*width slide) (mm^2)	13.00	13.00
residence time (hours)	5.56	1.11
linear velocity (m s^{-1}) [calculated using volumetric flow rate/surface area]	7.69E-05	3.85E-04
linear velocity (m s^{-1})	7.81E-05	1.88E-05

Flow rate used for Column attachment studies were based on Column studies previously conducted at the Centre for Bioprocess Engineering Research (CeBER), which in turn were based in industrial heap bioleach operation specifications. The specifications for the Column work are summarized in Table F1.

Industrial heaps flow rate = 5 L m^{-2} hr^{-1}

Table F1: CeBER column reactor specifications

Column diameter (cm)	10
Working length (cm)	32
Surface area column (cm^2)	78
Volume cylinder (cm^3)	2513
Ore loading (kg)	4
Residence time (hours)	42
Flow rate (ml min^{-1})	0.7
Flow rate (ml $hour^{-1}$)	40
Linear velocity (m sec^{-1})	1.41E-06

Appendix G: Examination of fluid flow in the column reactor

G1: Calculation of Reynolds number in the particle-packed column reactor

The dimensionless Reynolds number is used to characterize flow regimes and provides an indication of whether or not the fluid flow is laminar within the column. The Reynolds number makes use of the following fluid properties: density and viscosity, velocity and a characteristic length or characteristic dimension. For fluid flow through a pipe; a Reynolds number less than 2100 is indicative of fluid flow within the laminar region (Felder R. M., Rousseau R. W., Elementary principles of chemical processes 3rd Edition, 1999, John Wiley & Sons, Inc Danvers MA, USA). Conversely if the Reynolds number is greater than 2100, the fluid flow through the column is described as turbulent producing random eddies, vortices and other flow instabilities.

The Reynolds number is provided by the equation below.

$$\mathrm{Re} = \frac{\rho u l}{\mu} \qquad \text{Eqn G1}$$

Where:

ρ = density of water at 20° C (kg m-^{3})
u = fluid velocity m s^{-1}
l = length of the column (m)
μ = viscosity of water at 20° C (N s m^{-2}) x 10^{-3}

$$\mathrm{Re} = \frac{998.2 * 1.41E-06 * 0.19}{1.002E-03}$$
$$= 0.266884$$

The Reynolds number is less than 2100, therefore it can be assumed that fluid flow through the column is laminar.

G2: Calculation of the Peclet number (the dispersion model) in the particle-packed column reactor

Perfect plug flow was examined for operating conditions of the column reactor by considering the Peclet number and the dispersion model applicable to turbulent flow in pipes, laminar flow in long tubes and packed beds (Levenspiel O, *The chemical reactor omnibook*, Oregon State University Corvallis OR 97331, Chapters 63 and 64). Calculating Peclet number in packed column reactors provides an indication of the level of dispersion within the reactor and thus the level of deviation from perfect plug flow. The Peclet number is a dimensionless number and is related to the dispersion co-efficient, the fluid velocity in the column and the dimensions (length) of the column.

The Peclet number for fluid flow through a packed column is given in the equation below.

$$Pe_L = \frac{D}{u * L} \qquad \text{Eqn G2}$$

Where:

D = dispersion co-efficient ($m^2\ s^{-1}$)

u = fluid velocity ($m\ s^{-1}$)

L = length of the column (m)

The coefficient of dispersion (D) evaluated by recording the shape of the tracer curve as it passes the exit of the vessel. In particular, the mean time of passage is measured ($\bar{t}$), as well as the variance (σ^2), given by the equations below for data collected via mixed cup readings at equal time increments (Levenspiel O, *The chemical reactor omnibook*, Oregon State University Corvallis OR 97331, Chapter 63.5).

$$\bar{t} = \frac{\sum_{i=1}^{n} t_i C_i}{\sum_{i=1}^{n} C_i} \qquad \text{Eqn G3}$$

$$\sigma^2 = \frac{\sum_{i=1}^{n} t_i^2 C_i}{\sum_{i=1}^{n} C_i} - (\bar{t})^2 \qquad \text{Eqn G4}$$

The measures $\bar{t}$ and σ^2 are directly related to by theory to the dispersion coefficient (D) and the Peclet number $(\frac{D}{u * L})$. Chapter 64.5 of Levenspiel's *omnibook* graphically depicts how the spread of the tracer curve is dependant on the components of the Peclet number $(\frac{D}{u * L})$.

The Peclet number can be determined from the variance through the use of the following equation:

$$\sigma_\theta^2 = \frac{\sigma_t^2}{\left(\bar{t}\right)^2} \quad = 2\left(\frac{D}{u*L}\right)$$ Eqn G5

(Levenspiel O, *The chemical reactor omnibook*, Oregon State University Corvallis OR 97331, Chapter 64.4)

$\bar{t}$ and σ^2 where determined in excel using residence time distribution data collected via mixed cup readings at equal time increments and equations G3 and G4 above.

- $\bar{t}$ was approximately 55 minutes
- σ_t^2 was approximately 181
- σ_θ^2 was approximately 0.0604

Using equation G5 above to solve for the Peclet number ($P_{eL} = \frac{D}{u*L}$):

$$P_{eL} = \frac{\sigma_\theta^2}{2}$$

= 0.03

A Peclet number less than 0.01 is indicative of a small deviation level of dispersion and thus a small deviation from perfect plug flow. Conversely, systems yielding a Peclet number greater than 0.01 implies deviation from perfect plug flow (Levenspiel O, *The chemical reactor omnibook*, Oregon State University Corvallis OR 97331, Chapter 64.4).

The Peclet number was calculated under the conditions of this study and was determined to be 0.03 which is greater than 0.01. This indicates dispersion within the column and thus deviation from perfect plug flow. The fluid velocity and dimensions of the column reactor for this study deo not warrant perfect plug flow. For perfect plug flow to be attained with the dimensions of the column used here, the fluid velocity would have to be markedly increased. Perfect plug flow within the reactor was thus not assumed for this study.

u (m/s)	1.41E-06	Initial pH	7.18
L (m)	0.19	Initial [H+]concentration(mol/L)	6.61E-08

time (mins)	**pH**	**[H +] conc. (mol/L)**	**t_i*C_i**	**ti^2*C_i**	$\bar{t}$ (from Eqn G3)	σ_t^2 (from Eqn G4)
1	7.18	6.61E-08	6.61E-08	6.61E-08	54.7	180.8
2	7.17	6.76E-08	1.35E-07	2.70E-07		
3	7.15	7.08E-08	2.12E-07	6.37E-07		
4	7.14	7.24E-08	2.90E-07	1.16E-06		
5	7.1	7.94E-08	3.97E-07	1.99E-06		
6	7.09	8.13E-08	4.88E-07	2.93E-06		
7	7.08	8.32E-08	5.82E-07	4.08E-06		
8	7.07	8.51E-08	6.81E-07	5.45E-06		
9	7.05	8.91E-08	8.02E-07	7.22E-06		
10	7.01	9.77E-08	9.77E-07	9.77E-06		
11	6.98	1.05E-07	1.15E-06	1.27E-05		
12	6.94	1.15E-07	1.38E-06	1.65E-05		
13	6.89	1.29E-07	1.67E-06	2.18E-05		
14	6.84	1.45E-07	2.02E-06	2.83E-05		
15	6.74	1.82E-07	2.73E-06	4.09E-05		
16	6.65	2.24E-07	3.58E-06	5.73E-05		
17	6.51	3.09E-07	5.25E-06	8.93E-05		
18	6.35	4.47E-07	8.04E-06	1.45E-04		
19	6.13	7.41E-07	1.41E-05	2.68E-04		
20	5.74	1.82E-06	3.64E-05	7.28E-04		
21	4.88	1.32E-05	2.77E-04	5.81E-03		
22	4.34	4.57E-05	1.01E-03	2.21E-02		
23	4.25	5.62E-05	1.29E-03	2.97E-02		
24	4.2	6.31E-05	1.51E-03	3.63E-02		
25	4.14	7.24E-05	1.81E-03	4.53E-02		
26	4.08	8.32E-05	2.16E-03	5.62E-02		
27	4	1.00E-04	2.70E-03	7.29E-02		
28	3.93	1.17E-04	3.29E-03	9.21E-02		
29	3.84	1.45E-04	4.19E-03	1.22E-01		
30	3.77	1.70E-04	5.09E-03	1.53E-01		
31	3.75	1.78E-04	5.51E-03	1.71E-01		
32	3.74	1.82E-04	5.82E-03	1.86E-01		
33	3.73	1.86E-04	6.14E-03	2.03E-01		
34	3.71	1.95E-04	6.63E-03	2.25E-01		
35	3.67	2.14E-04	7.48E-03	2.62E-01		

36	3.65	2.24E-04	8.06E-03	2.90E-01		
37	3.62	2.40E-04	8.88E-03	3.28E-01		
38	3.61	2.45E-04	9.33E-03	3.54E-01		
39	3.6	2.51E-04	9.80E-03	3.82E-01		
40	3.59	2.57E-04	1.03E-02	4.11E-01		
41	3.58	2.63E-04	1.08E-02	4.42E-01		
42	3.57	2.69E-04	1.13E-02	4.75E-01		
43	3.56	2.75E-04	1.18E-02	5.09E-01		
44	3.55	2.82E-04	1.24E-02	5.46E-01		
45	3.54	2.88E-04	1.30E-02	5.84E-01		
46	3.53	2.95E-04	1.36E-02	6.24E-01		
47	3.51	3.09E-04	1.45E-02	6.83E-01		
48	3.5	3.16E-04	1.52E-02	7.29E-01		
49	3.49	3.24E-04	1.59E-02	7.77E-01		
50	3.47	3.39E-04	1.69E-02	8.47E-01		
51	3.45	3.55E-04	1.81E-02	9.23E-01		
52	3.41	3.89E-04	2.02E-02	1.05E+00		
53	3.38	4.17E-04	2.21E-02	1.17E+00		
54	3.33	4.68E-04	2.53E-02	1.36E+00		
55	3.3	5.01E-04	2.76E-02	1.52E+00		
56	3.26	5.50E-04	3.08E-02	1.72E+00		
57	3.26	5.50E-04	3.13E-02	1.79E+00		
58	3.27	5.37E-04	3.11E-02	1.81E+00		
59	3.28	5.25E-04	3.10E-02	1.83E+00		
60	3.29	5.13E-04	3.08E-02	1.85E+00		
61	3.3	5.01E-04	3.06E-02	1.86E+00		
62	3.31	4.90E-04	3.04E-02	1.88E+00		
63	3.32	4.79E-04	3.02E-02	1.90E+00		
64	3.33	4.68E-04	2.99E-02	1.92E+00		
65	3.34	4.57E-04	2.97E-02	1.93E+00		
66	3.35	4.47E-04	2.95E-02	1.95E+00		
67	3.36	4.37E-04	2.92E-02	1.96E+00		
68	3.37	4.27E-04	2.90E-02	1.97E+00		
69	3.38	4.17E-04	2.88E-02	1.98E+00		
70	3.4	3.98E-04	2.79E-02	1.95E+00		
71	3.44	3.63E-04	2.58E-02	1.83E+00		
72	3.5	3.16E-04	2.28E-02	1.64E+00		
73	3.56	2.75E-04	2.01E-02	1.47E+00		
74	3.65	2.24E-04	1.66E-02	1.23E+00		

75	3.77	1.70E-04	1.27E-02	9.55E-01		
76	3.89	1.29E-04	9.79E-03	7.44E-01		
77	3.91	1.23E-04	9.47E-03	7.29E-01		
78	4.07	8.51E-05	6.64E-03	5.18E-01		
79	4.21	6.17E-05	4.87E-03	3.85E-01		
80	4.3	5.01E-05	4.01E-03	3.21E-01		
81	4.37	4.27E-05	3.46E-03	2.80E-01		
82	4.55	2.82E-05	2.31E-03	1.90E-01		
83	4.8	1.58E-05	1.32E-03	1.09E-01		
84	5.13	7.41E-06	6.23E-04	5.23E-02		
85	5.37	4.27E-06	3.63E-04	3.08E-02		
86	5.64	2.29E-06	1.97E-04	1.69E-02		
87	5.72	1.91E-06	1.66E-04	1.44E-02		
88	5.8	1.58E-06	1.39E-04	1.23E-02		
89	5.86	1.38E-06	1.23E-04	1.09E-02		
90	5.91	1.23E-06	1.11E-04	9.97E-03		
91	5.93	1.17E-06	1.07E-04	9.73E-03		
92	5.96	1.10E-06	1.01E-04	9.28E-03		
93	5.99	1.02E-06	9.52E-05	8.85E-03		
94	6.02	9.55E-07	8.98E-05	8.44E-03		
95	6.04	9.12E-07	8.66E-05	8.23E-03		
96	6.06	8.71E-07	8.36E-05	8.03E-03		
97	6.1	7.94E-07	7.70E-05	7.47E-03		
98	6.12	7.59E-07	7.43E-05	7.29E-03		
99	6.19	6.46E-07	6.39E-05	6.33E-03		
100	6.24	5.75E-07	5.75E-05	5.75E-03		
101	6.28	5.25E-07	5.30E-05	5.35E-03		
102	6.3	5.01E-07	5.11E-05	5.21E-03		
103	6.34	4.57E-07	4.71E-05	4.85E-03		
104	6.39	4.07E-07	4.24E-05	4.41E-03		
105	6.43	3.72E-07	3.90E-05	4.10E-03		
106	6.46	3.47E-07	3.68E-05	3.90E-03		
107	6.5	3.16E-07	3.38E-05	3.62E-03		
108	6.52	3.02E-07	3.26E-05	3.52E-03		
109	6.56	2.75E-07	3.00E-05	3.27E-03		
110	6.57	2.69E-07	2.96E-05	3.26E-03		
111	6.59	2.57E-07	2.85E-05	3.17E-03		
112	6.62	2.40E-07	2.69E-05	3.01E-03		
113	6.64	2.29E-07	2.59E-05	2.93E-03		

114	6.69	2.04E-07	2.33E-05	2.65E-03		
115	6.71	1.95E-07	2.24E-05	2.58E-03		
116	6.74	1.82E-07	2.11E-05	2.45E-03		
117	6.75	1.78E-07	2.08E-05	2.43E-03		
118	6.77	1.70E-07	2.00E-05	2.36E-03		
119	6.79	1.62E-07	1.93E-05	2.30E-03		
120	6.81	1.55E-07	1.86E-05	2.23E-03		
121	6.82	1.51E-07	1.83E-05	2.22E-03		
122	6.84	1.45E-07	1.76E-05	2.15E-03		
123	6.86	1.38E-07	1.70E-05	2.09E-03		
124	6.87	1.35E-07	1.67E-05	2.07E-03		
125	6.89	1.29E-07	1.61E-05	2.01E-03		
126	6.91	1.23E-07	1.55E-05	1.95E-03		
127	6.92	1.20E-07	1.53E-05	1.94E-03		
128	6.93	1.17E-07	1.50E-05	1.92E-03		
129	6.94	1.15E-07	1.48E-05	1.91E-03		
130	6.95	1.12E-07	1.46E-05	1.90E-03		
131	6.96	1.10E-07	1.44E-05	1.88E-03		
132	6.97	1.07E-07	1.41E-05	1.87E-03		
133	6.98	1.05E-07	1.39E-05	1.85E-03		
134	6.99	1.02E-07	1.37E-05	1.84E-03		
135	7.01	9.77E-08	1.32E-05	1.78E-03		
136	7.02	9.55E-08	1.30E-05	1.77E-03		
137	7.03	9.33E-08	1.28E-05	1.75E-03		
138	7.04	9.12E-08	1.26E-05	1.74E-03		
139	7.05	8.91E-08	1.24E-05	1.72E-03		
140	7.06	8.71E-08	1.22E-05	1.71E-03		
141	7.07	8.51E-08	1.20E-05	1.69E-03		
142	7.08	8.32E-08	1.18E-05	1.68E-03		
143	7.09	8.13E-08	1.16E-05	1.66E-03		
144	7.1	7.94E-08	1.14E-05	1.65E-03		
145	7.11	7.76E-08	1.13E-05	1.63E-03		
146	7.15	7.08E-08	1.03E-05	1.51E-03		
147	7.18	6.61E-08	9.71E-06	1.43E-03		
	SUM	1.72E-02	9.43E-01	5.47E+01		

Appendix C3: Column attachment experimental raw data

Mineral System: PYRITE

Microorganism: ***A. ferrooxidans***

Growth history: Ore free cultured

Volume inoculums	Inoculum cell concentration (cells ml^{-1})			Time (mins) and/or Volume	Cell count on eluted samples			Cumulative no. cells eluted (cell no.)			Cumulative no. cells retained (cell no.)			Cells retained as a percentage of the inoculums (%)			Average (%)	Standard deviation
	Col 1	Col 2	Col 3		Col 1	Col 2	Col 3	Col 1	Col 2	Col 3	Col 1	Col 2	Col 3	Col 1	Col 2	Col 3		
10	2.66E+08	2.94E+08	2.81E+08	15	1	3	1	4.69E+06	1.41E+07	4.69E+06	2.65E+09	2.92E+09	2.81E+09	100%	100%	100%	100%	0.2%
				30	91	80	55	4.31E+08	3.89E+08	2.63E+08	2.23E+09	2.55E+09	2.55E+09	84%	87%	91%	85%	2.1%
				45	97	104	84	8.86E+08	8.77E+08	6.56E+08	1.77E+09	2.06E+09	2.16E+09	67%	70%	77%	68%	2.5%
				60	28	43	40	1.02E+09	1.08E+09	8.44E+08	1.64E+09	1.86E+09	1.97E+09	62%	63%	70%	63%	1.1%
				75	17	19	15	1.10E+09	1.17E+09	9.14E+08	1.56E+09	1.77E+09	1.90E+09	59%	60%	68%	59%	1.1%
				90	15	5	19	1.17E+09	1.19E+09	1.00E+09	1.49E+09	1.75E+09	1.81E+09	56%	59%	64%	58%	2.4%
				105	10	15	8	1.21E+09	1.26E+09	1.04E+09	1.44E+09	1.68E+09	1.77E+09	54%	57%	63%	56%	2.0%
				120	21	6	10	1.31E+09	1.29E+09	1.09E+09	1.34E+09	1.65E+09	1.73E+09	51%	56%	61%	53%	3.9%
				135	8	5	9	1.35E+09	1.31E+09	1.13E+09	1.31E+09	1.63E+09	1.68E+09	49%	55%	60%	52%	4.3%

Mineral System: PYRITE

Microorganism: *L. ferriphilum*

Growth history: Ore free cultured

Volume inoculums	Inoculum cell concentration (cells ml^{-1})			Time (mins) and/or Volume	Cell count on eluted samples			Cumulative no. cells eluted (cell no.)			Cumulative no. cells retained (cell no.)			Cells retained as a percentage of the inoculums (%)			Average (%)	Standard deviation
	Col 1	Col 2	Col 3		Col 1	Col 2	Col 3	Col 1	Col 2	Col 3	Col 1	Col 2	Col 3	Col 1	Col 2	Col 3		
10	1.17E+08	1.22E+08	1.61E+08	15	1	1	2	4.69E+06	4.69E+06	9.38E+06	1.17E+09	1.21E+09	1.60E+09	100%	100%	99%	100 %	0.0%
				30	13	17	10	6.56E+07	8.44E+07	5.63E+07	1.11E+09	1.13E+09	1.55E+09	94%	93%	97%	94%	0.9%
				45	29	24	34	2.02E+08	1.97E+08	2.16E+08	9.70E+08	1.02E+09	1.39E+09	83%	84%	87%	83%	0.7%
				60	34	25	27	3.61E+08	3.14E+08	3.42E+08	8.11E+08	9.05E+08	1.27E+09	69%	74%	79%	72%	3.6%
				75	16	13	19	4.36E+08	3.75E+08	4.31E+08	7.36E+08	8.44E+08	1.18E+09	63%	69%	73%	66%	4.5%
				90	7	13	12	4.69E+08	4.36E+08	4.88E+08	7.03E+08	7.83E+08	1.12E+09	60%	64%	70%	62%	3.0%
				105	5	9	6	4.92E+08	4.78E+08	5.16E+08	6.80E+08	7.41E+08	1.09E+09	58%	61%	68%	59%	2.0%
				120	5	8	4	5.16E+08	5.16E+08	5.34E+08	6.56E+08	7.03E+08	1.08E+09	56%	58%	67%	57%	1.2%
				135	3	4	4	5.30E+08	5.34E+08	5.53E+08	6.42E+08	6.84E+08	1.06E+09	55%	56%	66%	55%	1.0%

Mineral System: PYRITE

Microorganism: *S. metallicus*

Growth history: Ore free cultured

Volume inoculums used (ml)	Inoculum cell concentration (cells ml^{-1})			Time (mins) and/or Volume eluted	Cell count on eluted samples			Cumulative no. cells eluted (cell no.)			Cumulative no. cells retained (cell no.)			Cells retained as a percentage of the inoculums (%)			Average (%)	Standard deviation
	Col 1	Col 2	Col 3		Col 1	Col 2	Col 3	Col 1	Col 2	Col 3	Col 1	Col 2	Col 3	Col 1	Col 2	Col 3		
10	1.00E+08	1.00E+08	1.00E+08	15	45	50	50	2.11E+08	2.34E+08	2.34E+08	7.89E+08	7.66E+08	7.66E+08	79%	77%	77%	77%	1.4%
				30	48	48	40	4.36E+08	4.59E+08	4.22E+08	5.64E+08	5.41E+08	5.78E+08	56%	54%	58%	56%	1.9%
				45	41	39	35	6.28E+08	6.42E+08	5.86E+08	3.72E+08	3.58E+08	4.14E+08	37%	36%	41%	38%	2.9%
				60	31	22	31	7.73E+08	7.45E+08	7.31E+08	2.27E+08	2.55E+08	2.69E+08	23%	25%	27%	25%	2.1%
				75	16	8	17	8.48E+08	7.83E+08	8.11E+08	1.52E+08	2.17E+08	1.89E+08	15%	22%	19%	19%	3.3%
				90	8	8	14	8.86E+08	8.20E+08	8.77E+08	1.14E+08	1.80E+08	1.23E+08	11%	18%	12%	14%	3.5%
				105	10	6	14	9.33E+08	8.48E+08	9.42E+08	6.72E+07	1.52E+08	5.78E+07	7%	15%	6%	9%	5.2%
				120	8	5	6	9.70E+08	8.72E+08	9.70E+08	2.97E+07	1.28E+08	2.97E+07	3%	13%	3%	6%	5.7%
				135	4	5	2	9.89E+08	8.95E+08	9.80E+08	1.09E+07	1.05E+08	2.03E+07	1%	10%	2%	5%	5.2%

Mineral System: PYRITE

Microorganism: *A. ferrooxidans*

Growth history: Sulfide Mineral Adapted

Volume inoculums used (ml)	Inoculum cell concentration (cells ml^{-1})			Time (mins) and/or Volume eluted	Cell count on eluted samples			Cumulative no. cells eluted (cell no.)			Cumulative no. cells retained (cell no.)			Cells retained as a percentage of the inoculums (%)			Average (%)	Standard deviation
	Col 1	Col 2	Col 3		Col 1	Col 2	Col 3	Col 1	Col 2	Col 3	Col 1	Col 2	Col 3	Col 1	Col 2	Col 3		
10	3.08E+08	3.48E+08	3.84E+08	15	2	7	2	9.38E+06	3.28E+07	9.38E+06	3.07E+09	3.45E+09	3.83E+09	100%	99%	100%	100%	0.4%
				30	49	64	54	2.39E+08	3.33E+08	2.63E+08	2.84E+09	3.15E+09	3.58E+09	92%	90%	93%	92%	1.4%
				45	54	33	75	4.92E+08	4.88E+08	6.14E+08	2.59E+09	3.00E+09	3.23E+09	84%	86%	84%	85%	1.1%
				60	31	11	17	6.38E+08	5.39E+08	6.94E+08	2.44E+09	2.95E+09	3.15E+09	79%	85%	82%	82%	2.6%
				75	15	10	21	7.08E+08	5.86E+08	7.92E+08	2.37E+09	2.90E+09	3.05E+09	77%	83%	79%	80%	3.1%
				90	15	13	15	7.78E+08	6.47E+08	8.63E+08	2.30E+09	2.84E+09	2.98E+09	75%	81%	78%	78%	3.4%
				105	13	12	13	8.39E+08	7.03E+08	9.23E+08	2.24E+09	2.78E+09	2.92E+09	73%	80%	76%	76%	3.5%
				120	10	9	12	8.86E+08	7.45E+08	9.80E+08	2.19E+09	2.74E+09	2.86E+09	71%	79%	75%	75%	3.7%
				135	8	7	9	9.23E+08	7.78E+08	1.02E+09	2.15E+09	2.71E+09	2.82E+09	70%	78%	73%	74%	3.8%

Mineral System: PYRITE

Microorganism: *L. ferriphilum*

Growth history: Sulfide Mineral Adapted

Volume inoculums used (ml)	Inoculum cell concentration (cells ml^{-1})			Time (mins) and/or Volume eluted	Cell count on eluted samples			Cumulative no. cells eluted (cell no.)			Cumulative no. cells retained (cell no.)			Cells retained as a percentage of the inoculums (%)			Average (%)	Standard deviation
	Col 1	Col 2	Col 3		Col 1	Col 2	Col 3	Col 1	Col 2	Col 3	Col 1	Col 2	Col 3	Col 1	Col 2	Col 3		
10	3.11E+08	3.16E+08	3.31E+08	15	19	13	14	8.91E+07	6.09E+07	6.56E+07	3.02E+09	3.10E+09	3.25E+09	97%	98%	98%	98%	0.5%
				30	32	45	63	2.39E+08	2.72E+08	3.61E+08	2.87E+09	2.88E+09	2.95E+09	92%	91%	89%	91%	1.7%
				45	24	24	26	3.52E+08	3.84E+08	4.83E+08	2.76E+09	2.77E+09	2.83E+09	89%	88%	85%	87%	1.7%
				60	19	15	22	4.41E+08	4.55E+08	5.86E+08	2.67E+09	2.70E+09	2.73E+09	86%	86%	82%	85%	2.0%
				75	8	14	16	4.78E+08	5.20E+08	6.61E+08	2.63E+09	2.64E+09	2.65E+09	85%	84%	80%	83%	2.4%
				90	7	11	11	5.11E+08	5.72E+08	7.13E+08	2.60E+09	2.58E+09	2.60E+09	84%	82%	78%	81%	2.6%
				105	6	6	6	5.39E+08	6.00E+08	7.41E+08	2.57E+09	2.56E+09	2.57E+09	83%	81%	78%	80%	2.6%
				120	6	4	5	5.67E+08	6.19E+08	7.64E+08	2.54E+09	2.54E+09	2.55E+09	82%	80%	77%	80%	2.5%
				135	0	3	2	5.67E+08	6.33E+08	7.73E+08	2.54E+09	2.52E+09	2.54E+09	82%	80%	77%	79%	2.6%

Mineral System: PYRITE

Microorganism: ***S. metallicus***

Growth history: Sulfide Mineral Adapted

Volume inoculums	Inoculum cell concentration (cells ml^{-1})			Time (mins) and/or Volume	Cell count on eluted samples			Cumulative no. cells eluted (cell no.)			Cumulative no. cells retained (cell no.)			Cells retained as a percentage of the inoculums (%)			Average (%)	Standard deviation
	Col 1	Col 2	Col 3		Col 1	Col 2	Col 3	Col 1	Col 2	Col 3	Col 1	Col 2	Col 3	Col 1	Col 2	Col 3		
10	2.72E+08	2.14E+08	2.44E+08	15	0	1	0	0.00E+00	4.69E+06	0.00E+00	2.72E+09	2.14E+09	2.44E+09	100%	100%	100%	100%	0.1%
				30	11	11	9	5.16E+07	5.63E+07	4.22E+07	2.67E+09	2.08E+09	2.40E+09	98%	97%	98%	98%	0.5%
				45	92	83	81	4.83E+08	4.45E+08	4.22E+08	2.24E+09	1.70E+09	2.02E+09	82%	79%	83%	81%	1.9%
				60	112	113	129	1.01E+09	9.75E+08	1.03E+09	1.71E+09	1.17E+09	1.41E+09	63%	54%	58%	58%	4.3%
				75	57	62	80	1.28E+09	1.27E+09	1.40E+09	1.44E+09	8.75E+08	1.04E+09	53%	41%	43%	45%	6.6%
				90	23	24	47	1.38E+09	1.38E+09	1.62E+09	1.34E+09	7.63E+08	8.16E+08	49%	36%	33%	39%	8.5%
				105	21	22	25	1.48E+09	1.48E+09	1.74E+09	1.24E+09	6.59E+08	6.98E+08	46%	31%	29%	35%	9.2%
				120	19	18	25	1.57E+09	1.57E+09	1.86E+09	1.15E+09	5.75E+08	5.81E+08	42%	27%	24%	31%	9.9%
				135	18	16	15	1.65E+09	1.64E+09	1.93E+09	1.06E+09	5.00E+08	5.11E+08	39%	23%	21%	28%	9.9%

Mineral System: CHALCOPYRITE – pH 2.5

Microorganism: *A. ferrooxidans*

Growth history: Ore free cultured

Volume inoculums	Inoculum cell concentration (cells ml^{-1})			Time (mins) and/or Volume	Cell count on eluted samples			Cumulative no. cells eluted (cell no.)			Cumulative no. cells retained (cell no.)			Cells retained as a percentage of the inoculums (%)			Average (%)	Standard deviation
	Col 1	Col 2	Col 3		Col 1	Col 2	Col 3	Col 1	Col 2	Col 3	Col 1	Col 2	Col 3	Col 1	Col 2	Col 3		
10	1.88E+08	2.16E+08	2.23E+08	15	27	44	44	1.27E+08	2.06E+08	2.06E+08	1.75E+09	1.95E+09	2.03E+09	93%	90%	91%	91%	0.2%
				30	87	52	69	5.34E+08	4.50E+08	5.30E+08	1.34E+09	1.71E+09	1.70E+09	72%	79%	76%	78%	2.0%
				45	58	46	50	8.06E+08	6.66E+08	7.64E+08	1.07E+09	1.49E+09	1.47E+09	57%	69%	66%	67%	2.4%
				60	42	22	10	1.00E+09	7.69E+08	8.11E+08	8.72E+08	1.39E+09	1.42E+09	47%	64%	64%	64%	0.5%
				75	15	13	20	1.07E+09	8.30E+08	9.05E+08	8.02E+08	1.33E+09	1.33E+09	43%	62%	60%	61%	1.4%
				90	18	10	18	1.16E+09	8.77E+08	9.89E+08	7.17E+08	1.28E+09	1.25E+09	38%	59%	56%	58%	2.6%
				105	13	17	15	1.22E+09	9.56E+08	1.06E+09	6.56E+08	1.20E+09	1.18E+09	35%	56%	53%	54%	2.2%
				120	6	6	3	1.25E+09	9.84E+08	1.07E+09	6.28E+08	1.17E+09	1.16E+09	34%	54%	52%	53%	1.7%
				135	7	4	3	1.28E+09	1.00E+09	1.09E+09	5.95E+08	1.15E+09	1.15E+09	32%	53%	51%	52%	1.5%

Mineral System: CHALCOPYRITE – pH 1.5

Microorganism: *A. ferrooxidans*

Growth history: Ore free cultured

Volume inoculums used (ml)	Inoculum cell concentration (cells ml^{-1})			Time (mins) and/or Volume eluted	Cell count on eluted samples			Cumulative no. cells eluted (cell no.)			Cumulative no. cells retained (cell no.)			Cells retained as a percentage of the inoculums (%)			Average (%)	Standard deviation
	Col 1	Col 2	Col 3		Col 1	Col 2	Col 3	Col 1	Col 2	Col 3	Col 1	Col 2	Col 3	Col 1	Col 2	Col 3		
10.32	1.00E+09	1.00E+09		15	22	24		1.03E+08	1.13E+08		9.77E+08	9.74E+08		90%	90%		90%	0.6%
				30	34	39		2.63E+08	2.95E+08		8.18E+08	7.92E+08		76%	73%		74%	2.0%
				45	19	20		3.52E+08	3.89E+08		7.29E+08	6.98E+08		67%	64%		66%	2.3%
				60	21	16		4.50E+08	4.64E+08		6.30E+08	6.23E+08		58%	57%		58%	0.7%
				75	14	10		5.16E+08	5.11E+08		5.65E+08	5.76E+08		52%	53%		53%	0.5%
				90	4	9		5.34E+08	5.53E+08		5.46E+08	5.34E+08		51%	49%		50%	1.0%
				105	10	6		5.81E+08	5.81E+08		4.99E+08	5.06E+08		46%	47%		46%	0.2%
				120	1	2		5.86E+08	5.91E+08		4.94E+08	4.96E+08		46%	46%		46%	0.1%
				135	3	1		6.00E+08	5.95E+08		4.80E+08	4.92E+08		44%	45%		45%	0.5%
				165	1	2		6.09E+08	6.14E+08		4.71E+08	4.73E+08		44%	43%		44%	0.1%

				195	2	0		6.28E+08	6.14E+08		4.52E+08	4.73E+08		42%	43%		43%	1.2%
				225	1	1		6.38E+08	6.23E+08		4.43E+08	4.63E+08		41%	43%		42%	1.2%
				255	0	0		6.38E+08	6.23E+08		4.43E+08	4.63E+08		41%	43%		42%	1.2%
				285	0	0		6.38E+08	6.23E+08		4.43E+08	4.63E+08		41%	43%		42%	1.2%

Mineral System: CHALCOPYRITE

Microorganism: *L. ferriphilum*

Growth history: Ore free cultured

Volume inoculums used (ml)	Inoculum cell concentration (cells ml^{-1})			Time (mins) and/or Volume eluted	Cell count on eluted samples			Cumulative no. cells eluted (cell no.)			Cumulative no. cells retained (cell no.)			Cells retained as a percentage of the inoculums (%)			Average (%)	Standard deviation
	Col 1	Col 2	Col 3		Col 1	Col 2	Col 3	Col 1	Col 2	Col 3	Col 1	Col 2	Col 3	Col 1	Col 2	Col 3		
10	1.59E+08	1.78E+08	2.23E+08	15	2	1	3	9.38E+06	4.69E+06	1.41E+07	1.58E+09	1.78E+09	2.22E+09	99%	100%	99%	100%	0.2%
				30	4	5	13	2.81E+07	2.81E+07	7.50E+07	1.57E+09	1.75E+09	2.16E+09	98%	98%	97%	98%	1.0%
				45	31	44	79	1.73E+08	2.34E+08	4.45E+08	1.42E+09	1.55E+09	1.79E+09	89%	87%	80%	85%	4.7%
				60	60	76	73	4.55E+08	5.91E+08	7.88E+08	1.14E+09	1.19E+09	1.45E+09	71%	67%	65%	68%	3.4%
				75	82	71	67	8.39E+08	9.23E+08	1.10E+09	7.55E+08	8.58E+08	1.13E+09	47%	48%	51%	49%	1.7%
				90	36	36	43	1.01E+09	1.09E+09	1.30E+09	5.86E+08	6.89E+08	9.31E+08	37%	39%	42%	39%	2.5%
				105	17	22	31	1.09E+09	1.20E+09	1.45E+09	5.06E+08	5.86E+08	7.86E+08	32%	33%	35%	33%	1.7%
				120	14	11	15	1.15E+09	1.25E+09	1.52E+09	4.41E+08	5.34E+08	7.16E+08	28%	30%	32%	30%	2.2%
				135	13	10	7	1.21E+09	1.29E+09	1.55E+09	3.80E+08	4.88E+08	6.83E+08	24%	27%	31%	27%	3.4%

Mineral System: CHALCOPYRITE

Microorganism: *S. metallicus*

Growth history: Ore free cultured

Volume inoculums	Inoculum cell concentration (cells ml^{-1})			Time (mins) and/or	Cell count on eluted samples			Cumulative no. cells eluted (cell no.)			Cumulative no. cells retained (cell no.)			Cells retained as a percentage of the inoculums (%)			Average (%)	Standard deviation
	Col 1	Col 2	Col 3		Col 1	Col 2	Col 3	Col 1	Col 2	Col 3	Col 1	Col 2	Col 3	Col 1	Col 2	Col 3		
10	1.00E+08	1.00E+08	1.00E+08	15	1.00E+08	1.00E+08	1.00E+08	6.09E+07	4.69E+07	2.81E+07	9.39E+08	9.53E+08	9.72E+08	94%	95%	97%	95%	1.6%
				30	1.00E+08	1.00E+08	1.00E+08	4.45E+08	4.31E+08	4.13E+08	5.55E+08	5.69E+08	5.88E+08	55%	57%	59%	57%	1.6%
				45	1.00E+08	1.00E+08	1.00E+08	6.89E+08	7.88E+08	7.03E+08	3.11E+08	2.13E+08	2.97E+08	31%	21%	30%	27%	5.3%
				60	1.00E+08	1.00E+08	1.00E+08	8.25E+08	9.19E+08	8.44E+08	1.75E+08	8.13E+07	1.56E+08	18%	8%	16%	14%	5.0%
				75	1.00E+08	1.00E+08	1.00E+08	9.05E+08	9.70E+08	9.23E+08	9.53E+07	2.97E+07	7.66E+07	10%	3%	8%	7%	3.4%
				90	1.00E+08	1.00E+08	1.00E+08	9.47E+08	1.02E+09	9.75E+08	5.31E+07	-2.19E+07	2.50E+07	5%	-2%	3%	2%	3.8%
				105	1.00E+08	1.00E+08	1.00E+08	1.00E+09	1.08E+09	1.02E+09	-3.12E+06	-7.81E+07	-1.72E+07	0%	-8%	-2%	-3%	4.0%
				120	1.00E+08	1.00E+08	1.00E+08	1.08E+09	1.13E+09	1.05E+09	-7.81E+07	-1.34E+08	-4.53E+07	-8%	-13%	-5%	-9%	4.5%
				135	1.00E+08	1.00E+08	1.00E+08	1.15E+09	1.19E+09	1.09E+09	-1.48E+08	-1.91E+08	-8.75E+07	-15%	-19%	-9%	-14%	5.2%

Mineral System: CHALCOPYRITE

Microorganism: *A. ferrooxidans*

Growth history: Sulfide Mineral Adapted

Volume inoculums	Inoculum cell concentration (cells ml^{-1})			Time (mins) and/or Volume	Cell count on eluted samples			Cumulative no. cells eluted (cell no.)			Cumulative no. cells retained (cell no.)			Cells retained as a percentage of the inoculums (%)			Average (%)	Standard deviation
	Col 1	Col 2	Col 3		Col 1	Col 2	Col 3	Col 1	Col 2	Col 3	Col 1	Col 2	Col 3	Col 1	Col 2	Col 3		
10	3.00E+08	3.50E+08	3.44E+08	15	0	0	0	0.00E+00	0.00E+00	0.00E+00	3.00E+09	3.50E+09	3.44E+09	100%	100%	100%	100%	0.0%
				30	2	3	3	9.38E+06	1.41E+07	1.41E+07	2.99E+09	3.49E+09	3.42E+09	100%	100%	100%	100%	0.1%
				45	40	50	23	1.97E+08	2.48E+08	1.22E+08	2.80E+09	3.25E+09	3.32E+09	93%	93%	96%	94%	1.9%
				60	215	251	232	1.20E+09	1.43E+09	1.21E+09	1.80E+09	2.08E+09	2.23E+09	60%	59%	65%	61%	3.0%
				75	121	156	215	1.77E+09	2.16E+09	2.22E+09	1.23E+09	1.34E+09	1.22E+09	41%	38%	36%	38%	2.7%
				90	59	55	64	2.05E+09	2.41E+09	2.52E+09	9.52E+08	1.09E+09	9.20E+08	32%	31%	27%	30%	2.7%
				105	25	30	36	2.17E+09	2.55E+09	2.69E+09	8.34E+08	9.45E+08	7.52E+08	28%	27%	22%	26%	3.2%
				120	17	19	23	2.25E+09	2.64E+09	2.79E+09	7.55E+08	8.56E+08	6.44E+08	25%	24%	19%	23%	3.5%
				135	7	7	14	2.28E+09	2.68E+09	2.86E+09	7.22E+08	8.23E+08	5.78E+08	24%	24%	17%	21%	4.0%

Mineral System: CHALCOPYRITE

Microorganism: *L. ferriphilum*

Growth history: Sulfide Mineral Adapted

Volume inoculums	Inoculum cell concentration (cells ml^{-1})			Time (mins) and/or Volume	Cell count on eluted samples			Cumulative no. cells eluted (cell no.)			Cumulative no. cells retained (cell no.)			Cells retained as a percentage of the inoculums (%)			Average (%)	Standard deviation
	Col 1	Col 2	Col 3		Col 1	Col 2	Col 3	Col 1	Col 2	Col 3	Col 1	Col 2	Col 3	Col 1	Col 2	Col 3		
10	3.23E+08	3.20E+08	3.25E+08	15	22	27	31	1.03E+08	1.27E+08	1.45E+08	3.13E+09	3.08E+09	3.10E+09	97%	96%	96%	96%	0.6%
				30	141	153	133	7.64E+08	8.44E+08	7.69E+08	2.47E+09	2.36E+09	2.48E+09	76%	74%	76%	75%	1.6%
				45	32	39	31	9.14E+08	1.03E+09	9.14E+08	2.32E+09	2.18E+09	2.34E+09	72%	68%	72%	71%	2.2%
				60	21	22	22	1.01E+09	1.13E+09	1.02E+09	2.22E+09	2.07E+09	2.23E+09	69%	65%	69%	67%	2.3%
				75	12	19	17	1.07E+09	1.22E+09	1.10E+09	2.17E+09	1.98E+09	2.15E+09	67%	62%	66%	65%	2.7%
				90	13	12	13	1.13E+09	1.28E+09	1.16E+09	2.10E+09	1.93E+09	2.09E+09	65%	60%	64%	63%	2.6%
				105	13	11	13	1.19E+09	1.33E+09	1.22E+09	2.04E+09	1.88E+09	2.03E+09	63%	59%	63%	61%	2.5%
				120	10	11	9	1.24E+09	1.38E+09	1.26E+09	2.00E+09	1.83E+09	1.99E+09	62%	57%	61%	60%	2.6%
				135	10	10	11	1.28E+09	1.43E+09	1.31E+09	1.95E+09	1.78E+09	1.94E+09	60%	56%	60%	58%	2.6%

Mineral System: CHALCOPYRITE

Microorganism: ***S. metallicus***

Growth history: Sulfide Mineral Adapted

Volume inoculums	Inoculum cell concentration (cells ml^{-1})			Time (mins) and/or	Cell count on eluted samples			Cumulative no. cells eluted (cell no.)			Cumulative no. cells retained (cell no.)			Cells retained as a percentage of the inoculums (%)			Average (%)	Standard deviation
	Col 1	Col 2	Col 3		Col 1	Col 2	Col 3	Col 1	Col 2	Col 3	Col 1	Col 2	Col 3	Col 1	Col 2	Col 3		
10	1.22E+08	1.23E+08	1.36E+08	15	2	3	1	9.38E+06	1.41E+07	4.69E+06	1.21E+09	1.22E+09	1.35E+09	99%	99%	100%	99%	0.4%
				30	43	23	20	2.11E+08	1.22E+08	9.84E+07	1.01E+09	1.11E+09	1.26E+09	83%	90%	93%	89%	5.2%
				45	69	64	51	5.34E+08	4.22E+08	3.38E+08	6.84E+08	8.13E+08	1.02E+09	56%	66%	75%	66%	9.5%
				60	91	97	71	9.61E+08	8.77E+08	6.70E+08	2.58E+08	3.58E+08	6.89E+08	21%	29%	51%	34%	15.3%
				75	66	54	62	1.27E+09	1.13E+09	9.61E+08	-5.16E+07	1.05E+08	3.98E+08	-4%	8%	29%	11%	16.9%
				90	41	47	61	1.46E+09	1.35E+09	1.25E+09	-2.44E+08	-1.16E+08	1.13E+08	-20%	-9%	8%	-7%	14.3%
				105	19	26	13	1.55E+09	1.47E+09	1.31E+09	-3.33E+08	-2.38E+08	5.16E+07	-27%	-19%	4%	-14%	16.1%
				120	12	9	13	1.61E+09	1.51E+09	1.37E+09	-3.89E+08	-2.80E+08	-9.38E+06	-32%	-23%	-1%	-18%	16.0%
				135	9	8	9	1.65E+09	1.55E+09	1.41E+09	-4.31E+08	-3.17E+08	-5.16E+07	-35%	-26%	-4%	-22%	16.2%

Mineral System: LOW-GRADE CHALCOPYRITE CONTAINING MINERAL ORE

Microorganism: ***A. ferrooxidans***

Growth history: Ore free cultured

Volume inoculums	**Inoculum cell concentration (cells ml^{-1})**			**Time (mins) and/or Volume**	**Cell count on eluted samples**			**Cumulative no. cells eluted (cell no.)**			**Cumulative no. cells retained (cell no.)**			**Cells retained as a percentage of the inoculums (%)**			**Average (%)**	**Standard deviation**
	Col 1	Col 2	Col 3		Col 1	Col 2	Col 3	Col 1	Col 2	Col 3	Col 1	Col 2	Col 3	Col 1	Col 2	Col 3		
10	1.59E+08	1.47E+08	1.73E+08	15	4	2	3	1.88E+07	9.38E+06	1.41E+07	1.86E+09	2.15E+09	2.22E+09	99%	100%	99%	99%	0.4%
				30	1	6	2	2.34E+07	3.75E+07	2.34E+07	1.85E+09	2.12E+09	2.21E+09	99%	98%	99%	99%	0.3%
				45	35	146	62	1.88E+08	7.22E+08	3.14E+08	1.69E+09	1.43E+09	1.92E+09	90%	67%	86%	78%	16.6%
				60	121	113	149	7.55E+08	1.25E+09	1.01E+09	1.12E+09	9.05E+08	1.22E+09	60%	42%	55%	51%	12.6%
				75	125	79	79	1.34E+09	1.62E+09	1.38E+09	5.34E+08	5.34E+08	8.52E+08	29%	25%	38%	27%	2.6%
				90	35	33	37	1.50E+09	1.78E+09	1.56E+09	3.70E+08	3.80E+08	6.78E+08	20%	18%	30%	19%	1.5%
				105	23	19	20	1.61E+09	1.87E+09	1.65E+09	2.63E+08	2.91E+08	5.84E+08	14%	13%	26%	14%	0.4%
				120	23	10	16	1.72E+09	1.91E+09	1.73E+09	1.55E+08	2.44E+08	5.09E+08	8%	11%	23%	10%	2.2%
				135	17	3	5	1.80E+09	1.93E+09	1.75E+09	7.50E+07	2.30E+08	4.86E+08	4%	11%	22%	7%	4.7%

Mineral System: LOW-GRADE CHALCOPYRITE CONTAINING MINERAL ORE

Microorganism: ***L. ferriphilum***

Growth history: Ore free cultured

Volume inoculums	Inoculum cell concentration (cells ml^{-1})			Time (mins) and/or Volume	Cell count on eluted samples			Cumulative no. cells eluted (cell no.)			Cumulative no. cells retained (cell no.)			Cells retained as a percentage of the inoculums (%)			Average (%)	Standard deviation
	Col 1	Col 2	Col 3		Col 1	Col 2	Col 3	Col 1	Col 2	Col 3	Col 1	Col 2	Col 3	Col 1	Col 2	Col 3		
10	1.17E+08	1.44E+08	1.50E+08	15	3	3	1	1.41E+07	1.41E+07	4.69E+06	1.16E+09	1.42E+09	1.50E+09	99%	99%	100%	99%	0.2%
				30	4	3	3	3.28E+07	2.81E+07	1.88E+07	1.14E+09	1.41E+09	1.48E+09	97%	98%	99%	98%	0.6%
				45	5	13	12	5.63E+07	8.91E+07	7.50E+07	1.12E+09	1.35E+09	1.43E+09	95%	94%	95%	95%	1.0%
				60	46	58	43	2.72E+08	3.61E+08	2.77E+08	9.00E+08	1.08E+09	1.22E+09	77%	75%	82%	78%	1.3%
				75	30	41	38	4.13E+08	5.53E+08	4.55E+08	7.59E+08	8.84E+08	1.05E+09	65%	62%	70%	65%	2.3%
				90	33	40	34	5.67E+08	7.41E+08	6.14E+08	6.05E+08	6.97E+08	8.86E+08	52%	48%	59%	53%	2.2%
				105	27	33	28	6.94E+08	8.95E+08	7.45E+08	4.78E+08	5.42E+08	7.55E+08	41%	38%	50%	43%	2.2%
				120	20	22	24	7.88E+08	9.98E+08	8.58E+08	3.84E+08	4.39E+08	6.42E+08	33%	31%	43%	35%	1.6%
				135	15	18	16	8.58E+08	1.08E+09	9.33E+08	3.14E+08	3.55E+08	5.67E+08	27%	25%	38%	26%	1.5%

Mineral System: LOW-GRADE CHALCOPYRITE CONTAINING MINERAL ORE

Microorganism: *A. ferrooxidans*

Growth history: Sulfide Mineral Adapted

Volume inoculums	Inoculum cell concentration (cells ml^{-1})			Time (mins) and/or Volume	Cell count on eluted samples			Cumulative no. cells eluted (cell no.)			Cumulative no. cells retained (cell no.)			Cells retained as a percentage of the inoculums (%)			Average (%)	Standard deviation
	Col 1	Col 2	Col 3		Col 1	Col 2	Col 3	Col 1	Col 2	Col 3	Col 1	Col 2	Col 3	Col 1	Col 2	Col 3		
10	3.08E+08	3.52E+08	3.28E+08	15	2	2	4	9.38E+06	9.38E+06	1.88E+07	3.07E+09	3.51E+09	3.26E+09	100%	100%	99%	100%	0.2%
				30	164	177	209	7.78E+08	8.39E+08	9.98E+08	2.30E+09	2.68E+09	2.28E+09	75%	76%	70%	73%	3.5%
				45	155	98	133	1.50E+09	1.30E+09	1.62E+09	2.01E+09	1.98E+09	1.66E+09	57%	60%	51%	56%	5.0%
				60	70	67	75	1.83E+09	1.61E+09	1.97E+09	1.68E+09	1.67E+09	1.31E+09	48%	51%	40%	46%	5.7%
				75	61	54	64	2.12E+09	1.87E+09	2.27E+09	1.40E+09	1.42E+09	1.01E+09	40%	43%	31%	38%	6.4%
				90	42	33	44	2.32E+09	2.02E+09	2.48E+09	1.20E+09	1.26E+09	8.02E+08	34%	38%	24%	32%	7.2%
				105	23	25	32	2.42E+09	2.14E+09	2.63E+09	1.09E+09	1.14E+09	6.52E+08	31%	35%	20%	29%	7.8%
				120	14	15	19	2.49E+09	2.21E+09	2.72E+09	1.03E+09	1.07E+09	5.63E+08	29%	33%	17%	26%	8.2%
				135	13	11	8	2.55E+09	2.26E+09	2.76E+09	9.66E+08	1.02E+09	5.25E+08	27%	31%	16%	25%	7.9%

Mineral System: LOW-GRADE CHALCOPYRITE CONTAINING MINERAL ORE

Microorganism: *L. ferriphilum*

Growth history: Sulfide Mineral Adapted

Volume inoculums	Inoculum cell concentration (cells ml^{-1})			Time (mins) and/or Volume	Cell count on eluted samples			Cumulative no. cells eluted (cell no.)			Cumulative no. cells retained (cell no.)			Cells retained as a percentage of the inoculums (%)			Average (%)	Standard deviation
	Col 1	Col 2	Col 3		Col 1	Col 2	Col 3	Col 1	Col 2	Col 3	Col 1	Col 2	Col 3	Col 1	Col 2	Col 3		
10	2.19E+08	2.63E+08	2.77E+08	15	0	1	1	0.00E+00	4.69E+06	4.69E+06	2.19E+09	2.62E+09	2.76E+09	100%	100%	100%	100%	0.1%
				30	1	10	15	4.69E+06	5.16E+07	7.50E+07	2.18E+09	2.57E+09	2.69E+09	100%	98%	97%	98%	1.3%
				45	66	102	112	3.14E+08	5.30E+08	6.00E+08	1.87E+09	2.10E+09	2.17E+09	86%	80%	78%	81%	3.9%
				60	76	66	80	6.70E+08	8.39E+08	9.75E+08	1.52E+09	1.79E+09	1.79E+09	69%	68%	65%	67%	2.4%
				75	48	31	38	8.95E+08	9.84E+08	1.15E+09	1.29E+09	1.64E+09	1.61E+09	59%	63%	58%	60%	2.2%
				90	23	17	31	1.00E+09	1.06E+09	1.30E+09	1.18E+09	1.56E+09	1.47E+09	54%	59%	53%	56%	3.4%
				105	16	13	17	1.08E+09	1.13E+09	1.38E+09	1.11E+09	1.50E+09	1.39E+09	51%	57%	50%	53%	3.9%
				120	10	8	6	1.13E+09	1.16E+09	1.41E+09	1.06E+09	1.46E+09	1.36E+09	49%	56%	49%	51%	4.0%
				135	4	3	4	1.14E+09	1.18E+09	1.43E+09	1.04E+09	1.45E+09	1.34E+09	48%	55%	48%	50%	4.1%

Mineral System: QUARTZ (CONTROL MINERAL)

Microorganism: ***A. ferrooxidans***

Growth history: Ore free cultured

Volume inoculums	Inoculum cell concentration (cells ml^{-1})			Time (mins) and/or Volume	Cell count on eluted samples			Cumulative no. cells eluted (cell no.)			Cumulative no. cells retained (cell no.)			Cells retained as a percentage of the inoculums (%)			Average (%)	Standard deviation
	Col 1	Col 2	Col 3		Col 1	Col 2	Col 3	Col 1	Col 2	Col 3	Col 1	Col 2	Col 3	Col 1	Col 2	Col 3		
10	2.52E+08	2.64E+08	2.83E+08	15	1	2	2	4.69E+06	9.38E+06	9.38E+06	2.51E+09	2.63E+09	2.82E+09	100%	100%	100%	100%	0.1%
				30	9	13	4	4.69E+07	7.03E+07	2.81E+07	2.47E+09	2.57E+09	2.80E+09	98%	97%	99%	98%	0.6%
				45	52	85	60	2.91E+08	4.69E+08	3.09E+08	2.23E+09	2.17E+09	2.52E+09	88%	82%	89%	85%	4.4%
				60	143	161	137	9.61E+08	1.22E+09	9.52E+08	1.55E+09	1.42E+09	1.88E+09	62%	54%	66%	58%	5.8%
				75	76	60	67	1.32E+09	1.50E+09	1.27E+09	1.20E+09	1.14E+09	1.56E+09	48%	43%	55%	45%	3.3%
				90	35	22	32	1.48E+09	1.61E+09	1.42E+09	1.03E+09	1.03E+09	1.41E+09	41%	39%	50%	40%	1.4%
				105	16	10	20	1.56E+09	1.65E+09	1.51E+09	9.59E+08	9.86E+08	1.32E+09	38%	37%	47%	38%	0.6%
				120	15	7	8	1.63E+09	1.69E+09	1.55E+09	8.89E+08	9.53E+08	1.28E+09	35%	36%	45%	36%	0.5%
				135	7	5	2	1.66E+09	1.71E+09	1.56E+09	8.56E+08	9.30E+08	1.27E+09	34%	35%	45%	35%	0.8%

Mineral System: QUARTZ (CONTROL MINERAL)

Microorganism: *L. ferriphilum*

Growth history: Ore free cultured

Volume inoculums	Inoculum cell concentration (cells ml^{-1})			Time (mins) and/or	Cell count on eluted samples			Cumulative no. cells eluted (cell no.)			Cumulative no. cells retained (cell no.)			Cells retained as a percentage of the inoculums (%)			Average (%)	Standard deviation
	Col 1	Col 2	Col 3		Col 1	Col 2	Col 3	Col 1	Col 2	Col 3	Col 1	Col 2	Col 3	Col 1	Col 2	Col 3		
10	2.97E+08	2.91E+08	2.75E+08	15	3	2	1	1.41E+07	9.38E+06	4.69E+06	2.95E+09	2.90E+09	2.75E+09	100%	100%	100%	100%	0.2%
				30	17	19	13	9.38E+07	9.84E+07	6.56E+07	2.88E+09	2.81E+09	2.68E+09	97%	97%	98%	97%	0.5%
				45	26	31	34	2.16E+08	2.44E+08	2.25E+08	2.75E+09	2.66E+09	2.53E+09	93%	92%	92%	92%	0.6%
				60	57	51	80	4.83E+08	4.83E+08	6.00E+08	2.49E+09	2.42E+09	2.15E+09	84%	83%	78%	82%	3.1%
				75	72	78	75	8.20E+08	8.48E+08	9.52E+08	2.15E+09	2.06E+09	1.80E+09	72%	71%	65%	70%	3.7%
				90	42	41	43	1.02E+09	1.04E+09	1.15E+09	1.95E+09	1.87E+09	1.60E+09	66%	64%	58%	63%	4.1%
				105	31	33	32	1.16E+09	1.20E+09	1.30E+09	1.81E+09	1.71E+09	1.45E+09	61%	59%	53%	57%	4.3%
				120	22	21	19	1.27E+09	1.29E+09	1.39E+09	1.70E+09	1.61E+09	1.36E+09	57%	55%	49%	54%	4.2%
				135	21	15	21	1.36E+09	1.36E+09	1.49E+09	1.60E+09	1.54E+09	1.26E+09	54%	53%	46%	51%	4.5%

Mineral System: QUARTZ (CONTROL MINERAL)

Microorganism: *A. ferrooxidans*

Growth history: Sulfide Mineral Adapted

Volume inoculums	Inoculum cell concentration (cells ml^{-1})			Time (mins) and/or Volume	Cell count on eluted samples			Cumulative no. cells eluted (cell no.)			Cumulative no. cells retained (cell no.)			Cells retained as a percentage of the inoculums (%)			Average (%)	Standard deviation
	Col 1	Col 2	Col 3		Col 1	Col 2	Col 3	Col 1	Col 2	Col 3	Col 1	Col 2	Col 3	Col 1	Col 2	Col 3		
10	2.66E+08	3.28E+08	3.02E+08	15	0	0	0	0.00E+00	0.00E+00	0.00E+00	2.66E+09	3.28E+09	3.02E+09	100%	100%	100%	100%	0.0%
				30	1	0	0	4.69E+06	0.00E+00	0.00E+00	2.65E+09	3.28E+09	3.02E+09	100%	100%	100%	100%	0.1%
				45	36	25	32	1.73E+08	1.17E+08	1.50E+08	2.48E+09	3.16E+09	2.87E+09	93%	96%	95%	95%	1.5%
				60	161	180	196	9.28E+08	9.61E+08	1.07E+09	1.73E+09	2.32E+09	1.95E+09	65%	71%	65%	67%	3.4%
				75	150	161	201	1.63E+09	1.72E+09	2.01E+09	1.03E+09	1.57E+09	1.00E+09	39%	48%	33%	40%	7.3%
				90	93	39	59	2.07E+09	1.90E+09	2.29E+09	5.89E+08	1.38E+09	7.28E+08	22%	42%	24%	29%	11.0%
				105	33	33	21	2.22E+09	2.05E+09	2.39E+09	4.34E+08	1.23E+09	6.30E+08	16%	37%	21%	25%	11.1%
				120	11	12	7	2.27E+09	2.11E+09	2.42E+09	3.83E+08	1.17E+09	5.97E+08	14%	36%	20%	23%	11.1%
				135	5	3	5	2.30E+09	2.12E+09	2.44E+09	3.59E+08	1.16E+09	5.73E+08	14%	35%	19%	23%	11.3%

Mineral System: QUARTZ (CONTROL MINERAL)

Microorganism: *L. ferriphilum*

Growth history: Sulfide Mineral Adapted

Volume inoculums	**Inoculum cell concentration (cells ml^{-1})**			**Time (mins) and/or Volume**	**Cell count on eluted samples**			**Cumulative no. cells eluted (cell no.)**			**Cumulative no. cells retained (cell no.)**			**Cells retained as a percentage of the inoculums (%)**			**Average (%)**	**Standard deviation**
	Col 1	Col 2	Col 3		Col 1	Col 2	Col 3	Col 1	Col 2	Col 3	Col 1	Col 2	Col 3	Col 1	Col 2	Col 3		
10	1.88E+08	1.94E+08	1.92E+08	15	1	0	1	4.69E+06	0.00E+00	4.69E+06	1.87E+09	1.94E+09	1.92E+09	100%	100%	100%	100%	0.1%
				30	5	12	10	2.81E+07	5.63E+07	5.16E+07	1.85E+09	1.88E+09	1.87E+09	99%	97%	97%	98%	0.8%
				45	69	74	110	3.52E+08	4.03E+08	5.67E+08	1.52E+09	1.53E+09	1.35E+09	81%	79%	70%	77%	5.7%
				60	102	110	132	8.30E+08	9.19E+08	1.19E+09	1.05E+09	1.02E+09	7.36E+08	56%	53%	38%	49%	9.3%
				75	76	64	39	1.19E+09	1.22E+09	1.37E+09	6.89E+08	7.19E+08	5.53E+08	37%	37%	29%	34%	4.7%
				90	11	19	28	1.24E+09	1.31E+09	1.50E+09	6.38E+08	6.30E+08	4.22E+08	34%	33%	22%	29%	6.6%
				105	6	11	9	1.27E+09	1.36E+09	1.54E+09	6.09E+08	5.78E+08	3.80E+08	33%	30%	20%	27%	6.7%
				120	5	4	6	1.29E+09	1.38E+09	1.57E+09	5.86E+08	5.59E+08	3.52E+08	31%	29%	18%	26%	6.9%
				135	7	4	8	1.32E+09	1.40E+09	1.61E+09	5.53E+08	5.41E+08	3.14E+08	30%	28%	16%	25%	7.2%